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SPECIES STUDIES AND THE SPECIES PROBLEM[1]

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IN a recent symposium of the Linnean Society of London on "The Species Problem in Phanerogams"[2] the critical term was variously defined by participants. In absence of previous agreement it seems desirable, then, to state in what sense one uses it in one's own contribution.

For me the species problem is set by the existence of a diversity of organic forms in the world. It is the old question of the way in which they have come into being. Through biological literature for a very long time runs at least a thin thread of suggestion that for solution of the problem in this sense much may justly be expected from the study of species.[3] If they are the product of evolution, in the relations in which they stand to one another they should reveal the nature of its process.

Here, for safety's sake, one's first move should be to determine whether species living to-day are groups given by nature, *i.e.*, whether their bases are objective rather than subjective. And this, it may be observed, is not the question whether or no any universal criterion distin-

[1] Presented at a joint symposium on "The Species Problem," of the American Society of Zoologists and the American Genetics Society, Princeton University, December 30, 1935.

[2] See *Proc. Linn. Soc. Lond.*, 103–119, 1935.

[3] See C. Tate Regan, Report Brit. Assoc. Adv. Sci., Southampton meeting, p. 75, 1925; H. M. Hall, *Scientific Monthly*, 35, p. 289, 1932.

guishes species from variety. To his own satisfaction Darwin, after eight years' study of *Cirripedia,* answered that in the negative, and few fail to approve his decision. But both species and varieties may nevertheless be real, as cities and towns are both real, though no infallible mark distinguishes them.

The proposed inquiry is confined to living species. For choosing the present, however, rather than another geological horizon, there is no advantage except that our knowledge of existing floras and faunas is more complete than that we have of any preceding them. But in restricting the study to some single time-level a gain is made which is after the order of Mendel's, for example, when in his simpler experiments he confined his observations to single characteristics in the generations following a cross. By simplifying our problem through imposition of artificial limits we discover parts of a whole we might otherwise miss entirely, and may rest assured that whatever we find of truth will fit the remainder appearing first perhaps when such are dispensed with.

Such a study of species as is required to determine whether they are real, in the sense a preceding paragraph indicates, necessity has for another reason in a way compelled me to make, with results which may be summarized approximately as follows:

Among West Indian marine fishes males and females of one species are at present ten times listed as distinct sorts. Ten times kinds currently counted good species are distinguished by nothing save chameleonic color-phases passing in a moment in sorts known under earlier names. Thirty times the types are merely growth stages in life histories of species named previously from older or younger specimens. Regarding these 50 alleged species no dispute is possible. With such knowledge of the living organism as taxonomists often have no opportunity to acquire, their 50 names must be referred to synonymy without reservation.

More than 90 other names currently accepted must go the same way. Between the types upon which they are

based and types of species earlier named there is rarely any difference transgressing the bounds of individual variation in even a small sample from one locality. The usual source of error here is that authors of unwarranted new names have had too little or too poorly preserved material, or have been unacquainted at first hand with the types of earlier workers and for necessary comparisons have been forced to depend upon printed descriptions incomplete or inaccurate in detail.

To drop even 140 names from the current record of West Indian marine fishes is not enough. At least a few more must go, but the roll of synonyms is long enough to show how matters stand. For retention of roughly 20 per cent. of listed species of these fishes there is not the slightest justification in fact. This fraction is not set apart from others by peculiarities which are in my judgment merely less than "specific difference" should be. Their alleged peculiarities as species are entirely fictitious.

Now rejection of so many supposed species opens out notably the gaps between those remaining. Enrichment, too, of the definition of many species retained occurs in the course of revision, which without the remotest relation to what is called "lumping" may bring under a single specific name sexual forms or growth phases earlier counted different. Both processes make species seem much more distinct than was the case in the beginning. Revision also removes a disproportionate amount of what might earlier have been considered evidence of intergradation.

No one qualified by experience to pass independent judgment in the matter for a moment suggests that the boundaries of all species are ill-defined, that everywhere species intergrade with one another. But between males and females of one species whose sexual dimorphism develops late, between old and young of any species undergoing change with growth, between transient color-phases, between the well and poorly preserved, the well and poorly described of one species, there must of neces-

sity be intergrades. After each rectification, therefore, in any of the categories mentioned the examples of possible intergradation are a smaller fraction of the whole remaining. And there is another type of correction, which in effect is the same as these, though it involves striking no name from the list. This is made whenever a name is transferred from one synonymy to another in which it properly belongs, for here again in the unrevised list we have under different specific names two fractions of a single species between which intergrades must occur. In the material studied this transfer has been necessary in seven instances.

That actual species of marine fishes in the region from Bermuda to Florida, Panama and Brazil commonly intergrade to-day, that they are groups arbitrarily set apart from their contemporaries, are hypotheses for which the evidence grows less as one knows one's material better. They are, then, hypotheses one may reasonably assume must eventually be abandoned. They are in fact even now quite untenable. But ignorance of the living organism and inaccessibility of types are handicaps under which all taxonomy labors. It is therefore a fair conclusion that with like deceptive result like error in different degree mars the taxonomic record in all groups.

From this point, in conformity with evidence presented, it is assumed that what are justly called species are distinct entities, natural populations not commonly intergrading. In this case it is fairly to be inferred that in their internal structure as populations, in their relations to one another in space and time, they should, if intimately enough known, show how they were made.

This view of the matter, indeed, we all tacitly accept. From the fact that we observe species to be larger or smaller clusters of varieties,[4] which themselves seem "incipient species"; from the fact that in time we find primitive generalized forms preceding the specialized and degenerate; and, in space, endemism correlated with isolation, and endemic species of remote islands most resem-

[4] See W. B. Brierley, *Proc. Linn. Soc. Lond., tom. cit.*, figs. 2–3.

bling inhabitants of the continent from which prevailing winds and ocean currents might most readily have brought their ancestors, we all conclude without hesitation that species have been made by a process of evolution rather than by special creation.

Darwin compared species with respect to the areas they occupy,[5] but in no comprehensive way from lack of information. He knew, and we know accurately now, the areas occupied by few or no species. But this difficulty is readily enough overcome, as may next be shown, and being overcome will give opportunity to study species in a new relation.

The most peculiar, the most readily distinguishable species of any fauna or flora, are in general those of its monotypic genera. And, being most readily recognizable, they are as a class the first animals and plants of a country whose ranges are known with approximate accuracy. The recorded growth of knowledge of the ranges of British plants, as it is set down now for nearly a century in successive editions of the London Catalogue, makes the inference certain.

Of endemic monotypes among the flowering plants of New Zealand, for example, there are 57 species. Having grouped adjacent small counties and divided a few of the large, one has marked off on the map of that country about 60 units of nearly equal area and may with available records[6] find out to how many of them each of the 57 monotypes goes. The history of scientific exploration in New Zealand is still short, the record of its advance is nearly complete, and so it is possible to determine with no great error upon the average what was the time of first finding by scientific men of specimens of each of the species under consideration. It is a simple matter to check the results of the two inquiries, one against the other, with results which at the moment stand as follows:

Species at present recorded from 1 unit of area only are known on the average since 1888, from 2 units since

[5] See "Origin of Species," Chap. II, "Variation under Nature."

[6] See T. F. Cheeseman, "Manual of the New Zealand Flora," pp. xliv + 1163, ed. 2, 1925.

1863, from 3 to 5 units since 1860, from 6 to 10 units since 1848, from 11 to 21 units since 1828, 22 to 41 units since 1799, and from more than 41 units since 1788.

This is to say, new species occupying very small areas are occasionally found still, but those very widely disseminated in New Zealand were included in large part in collections made by Banks and Solander at the time of Cook's first voyage and the discovery of the islands, or were found by other early explorers. To generalize the statement, in any country the time of first finding of species inhabiting it is upon the average largely determined by the area they occupy there. Differences in the average area occupied by groups of species in the same general circle of affinity may therefore be investigated by way of the average time of first finding, as it is always possible to study an inaccessible primary by way of its accessible correlated variable.

Applying the suggestion, and making a study of the dependence of time of finding upon the size of the genus to which species belong, it appears that the species of small genera tend to be found late and those of large ones early. This statement, after the legitimate substitution of area for time, reads: *The species of small genera upon the average occupy small areas and those of large genera large ones.*

This is obviously a law of geographic distribution. It is, moreover, a law for the existence of which there seems to be only one explanation. The correlations it states, to recast them, are these: When the species in a genus are few they have upon the average limited tolerance of environmental conditions, and when they are many their tolerance of those conditions is wide. This means, I think, that ancestors which, if they had lived till the present, would have displayed limited tolerance of such conditions as their descendant species are compelled to endure to-day, have upon the whole produced species-descendants like themselves in their tolerance; as have those generic ancestors with many descendant species, whose average tolerance is wide.

In other words, the law of distribution is as it is, because evolution is a fact; and because in evolution like tends to beget like, and success in begetting descendant species depends upon the survival value of ancestral endowment and legacy. Study of species in this new relation confirms, then, what has been inferred from studying them in others earlier, and advances knowledge besides concerning a detail of the process by which they were made. It leaves it no hypothesis that natural selection is a factor in evolution, but exhibits it as a fact demonstrated by evidence which proves the occurrence of evolution itself.

This is not in accord, of course, with the view that variation and inheritance are in a class by themselves as factors in evolution. When heritable variation has occurred, say those of this mind, the new has arrived. These forget, however, that the new form is born of parents which have endured successfully such struggle for existence as there may be. Through that door natural selection enters into the process of evolution upon equal terms with variation: for what determines what shall vary determines in an effective way the outcome of variation.

But the main point at the moment lies in another direction. If species are entities formed by natural process, and with respect to area occupied are distributed according to law, then the process of evolution by which they were made is itself in some sense an orderly one. There can be no natural law of geographic distribution which does not entail the existence of a law of evolution.

That evolution does in fact occur according to law we have for years had evidence to show. No less is to be inferred from another relation in which species stand to one another. This is what Dr. J. C. Willis discovered, and which his hypothesis of "Age and Area" I must join others in rejecting was devised to explain. When in any great group of organisms the known genera are grouped according to the number of their own living species, the monotypic in the first class, the ditypic genera next, and

so on to the largest, their statistical distribution, expressed by the Willis curves, conforms rather closely to one system.[7]

These curves will not be discussed here in detail. Time does not permit it. I am mindful, however, of the fact that they are based upon records the accuracy of which I have myself impugned.

Inaccuracy, I take it, inaccuracy of the same general sort, mars the taxonomic record in all great groups, and in so far as it is of the same sort warps all Willis's curves in the same way. Its only effect in that case is to obscure the precise nature of the law, evidence for the existence of which is still conclusive.

The law of evolution is elsewhere stated[8] and for brevity's sake I pass to questions suiting better this general paper. Of these the first is, why evolution, from a statistical point of view, should conform to law.

The answer may be suggested in few words. Imagine one had seen, say, the efflorescence of the reptilian stock between Lower Carboniferous time and the height of the Mesozoic. One might say then with equal accuracy that one had followed through the evolution of the Reptilia or had seen a world-population of reptiles come into being. The terms are equivalent. The Mesozoic reptilian population, however, differed from those populations of flies or men, for example, whose increase, we know, under certain conditions conforms to one law. It was compound, they are simple populations. But it is almost inconceivable that simple populations should grow according to law and that compound populations, of which the simple are the units, should not differentiate according to law.

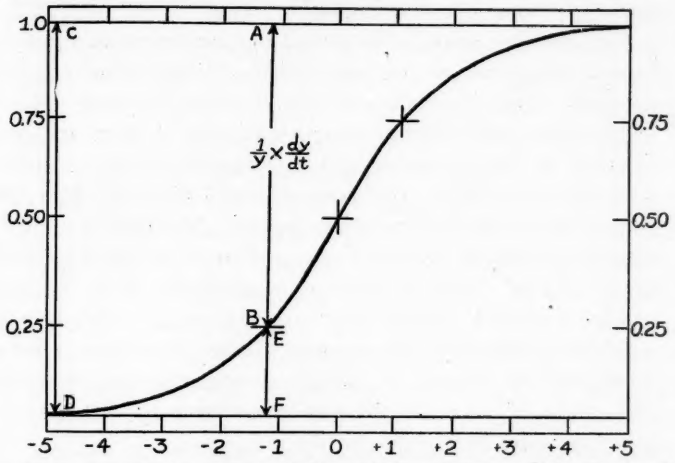
We must now consider more closely the law of growth of simple populations, for in it, remote as it may seem from species questions, is to be found a hint for solution of the species problem, at least from one point of view.

The law, first formulated by Verhulst in 1838 and re-

[7] See J. C. Willis, "Age and Area," Cambridge University Press, p. 187, 1922.

[8] W. H. Longley, *Anat. Rec.*, xliv, 241, 1929.

discovered by Pearl and Reed[9] in 1920, is expressed graphically in the accompanying figure. It is the law of



Curve of population growth, showing relation of the proportional rate of increase and attained fraction of the limit, when limited resources are maintained at one level by periodic renewal.

change in length of the vertical line AB, assumed to be moving from left to right with its foot upon the curve of population growth and its head at the level of the population's limit. But this line AB, representing the proportional rate of increase of the population, is equal to the difference in length of the lines CD and EF. CD, however, represents the momentary rate of increase in the minimal initial population growing in the virtual absence of checks imposed by its own numbers; and EF represents the attained fraction of the limiting population. The law may therefore be expressed in ordinary language as follows:

While periodic renewal maintains its limited resources in matter and energy sensibly at one level, a simple population so multiplies that the proportional increase anticipated in absence of checks fails in the same measure as the attained fraction of the limiting population grows.[10]

[9] R. Pearl and L. J. Reed, *Proc. Nat. Acad. Sci.*, 6, 275–288, 1920.

[10] Though it may seem so, this statement is not inconsistent with experimental results obtained by Pearl by a culture method first described in the

From this law interesting corollaries may be derived by inspection. Their nature will be the more readily apparent in a concrete example.

The limiting population of wild-type *Drosophila* is 212 flies to the standard half-pint bottle.[11] One hundred and six flies, then, in such a bottle constitute a population half-grown, and their proportional rate of increase, according to the terms of the law itself, would be short half the ideal rate. But the same 106 flies, put 53 each in two standard bottles, with due regard to age and sex, would constitute populations one-quarter grown, and their rate of increase after due partition of an appropriate juvenile population appropriately maintained would be short only one quarter of the ideal rate. So by doubling the volume of their universe the deficiency in their rate of increase, as compared with the ideal rate, is reduced by half.

Under the circumstances it is obvious that one needs only to call this deficiency a "pressure effect" in order to have derived from the law of increase in a simple population another law of populations, which in form is like Boyle's law.

Quarterly Review of Biology, vol. II, pp. 543–544, 1927. Under this "third" method by renewing at 3-day intervals the resources of the "universe" in which his increasing adult population of flies was growing, he kept matter and energy available to them practically at constant level in his lead-bottle. But his lag-bottles, increased in number by one each three days at the beginning of the experiment, for a time at least provided an "expanding universe" for the culture of larvae. As a result more imagoes were reared in the several-bottle universe reserved for larvae than was consistent with the limit imposed in the lead-bottle. Their transfer to the lead-bottle as they appeared should first have increased the adult population there more rapidly than computation would have led one to expect. This increase, abnormal from the point of view of a population growing in a one-unit universe, should then have entailed (according to Pearl's own experience) an abnormally abrupt diminution in the fecundity of the crowded flies, with perpetuation of oscillations in the empirical curve the experiment was yielding. Initial failure to impose upon the larval population a restraint commensurate with that imposed upon the adults, and not differences in the nutritional level in the lead-bottle under the third, as opposed to that in the single bottle of his second method, is responsible, I take it, for the strikingly different outcome under the two.

[11] See R. Pearl, "The Biology of Population Growth," Alfred A. Knopf, New York, p. 41, 1925.

It is clear, too, from the terms of the law of increase that if in two standard bottles two unknown numbers of flies, x and x_1, of the same stock, under standard culture conditions show the same deficiency in comparison with the ideal rate of increase, then $x = x_1$. And here, again, if the shortage be called a pressure effect, the law of populations deduced by inspection from the law of increase is in form like Avogadro's law.[12]

But from Boyle's and Avogadro's laws, empirically determined from study of gases, it was inferred at last that these are particulate systems composed of active units acting upon one another at random. One is led then by easy steps to ask one's self whether there may not be different kinds of kinetic system, *i.e.*, different kinds of particulate system, whose units agree only in the very abstract qualities of being active and acting upon one another at random, but differ in their secondary attributes. If such systems exist, is not the compound, or species population, one of them?

In fact, such systems as are suggested do exist. Normal gases are merely the simplest sort. Their units are ultramicroscopic, neither reproducing nor varying significantly. The intensity of the influence they exert upon one another is appropriately measured with a manometer, and when so measured at constant temperature under experimental conditions permits those results to be stated which go by the names of Boyle's and Avogadro's laws.

Solutions are equally kinetic systems, events in which are complicated by the presence of a solvent medium. Active, acting at random, ultramicroscopic, invariable, not reproducing, their units by their activity do not achieve results in all respects directly comparable with those of the molecules in a normal gas. Apparatus of a quite distinct sort is required to measure the intensity of their influence in terms of "osmotic pressure," before telltale relations in form like Boyle's and Avogadro's laws appear and declare solutions what they are.

[12] It should be observed that it is not from the form of the curve of population growth, but from the empirical data the curve summarizes, that these inferences are drawn.

Glowing gases are kinetic systems whose units are variable, though not all actually varying at once. But in the atoms composing the varying fraction, constantly maintained while external conditions are constant, electrons absorb quanta of energy and, if we adopt Bohr's view, leap to orbits more remote, or give up quanta of energy and return to less remote.

The variability of the units in these gases is for practical purposes a new attribute. It makes possible a new type of event in the system. If limited in its expression by the pressure in the system, its results must conform to a new law of gases; and this law must be one from which, if first of gas laws to be discovered, Boyle's and Avogadro's might have been deduced by inspection. For no law of gases including a factor for pressure is fully stated without factors for temperature and volume, and these must enter upon terms satisfying the laws of Boyle and Avogadro.

Returning now to biology, and shortly to species, it should be clear why simple populations, growing under terms specified, follow a law of increase from which laws of the form of Boyle's and Avogadro's may be deduced by inspection. They are systems of active units, acting at random, having a power of reproduction limited in its expression by restraints their own numbers impose. And the necessity of measuring influence in such populations as an effect upon the proportionate rate of increase, before laws resembling Boyle's and Avogadro's in form may be derived, is patent. It is only a special case under a general rule:

For each new sort of influence unit exerts upon unit from one kinetic system to the next its own fit mode of measurement must be employed before these instructive numerical relations come to light, which it appears are almost as commonly recurrent as the law of inverse squares, itself developed in the fields of sound, light, electricity, magnetism, *etc.*, but only by methods of measurement appropriate in each.

So the study of biological species leads directly to the

conclusion that their differentiation in every great group of organisms follows one law.[13] And search for the basis of law leads to recognition of a series of species of kinetic system, in all of which law results from the activity and random action of their units one upon another and their consequent tendency to attain a state of equilibrium under the conditions imposed upon them.

The law of evolution to which reference was earlier made is the law of differentiation of the most complex type recognized, the sort in which units at once reproduce and vary. It owes its precise form to the fact that the incidence of partial pressures upon fractions of the population endlessly varies at random, while the sum of the partial pressures itself approaches a limit fixed from the beginning.

Summary

Species are natural populations, upon the whole sharply distinct from one another. In internal structure and in their relations to one another in space and time they show that they were made by a process of evolution in which hereditary variation and natural selection are factors.

Evolution itself is the process of differentiation of a compound population. Compound populations differentiate according to law, as definitely as simple ones increase according to law under appropriate conditions.

The laws of populations, simple and compound, at bottom are laws of the same sort as laws of gases and solutions; and owe their existence fundamentally to the same cause. Gases, solutions, populations are systems of active units acting upon one another at random, differing from one another in secondary attributes, but tending, however disturbed, to return to equilibrium.[14]

[13] Preliminary statements, upon some points amplifying the present, may be found in *Nature* (London), Vol. 131, p. 863, 1933, and *Proc. Linn. Soc. Lond.*, April 6, 1933, or traced through these references.

[14] The relation of these facts to experimental programs for social betterment and the improbability of such succeeding as ignore the laws of populations will not escape the reflective student.

THE DISTRIBUTION OF PHYSIOLOGICAL AND CHEMICAL PECULIARITIES IN THE "NATURAL" GROUPS OF ORGANISMS[1]

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I HAVE been asked to contribute to this symposium as a physiologist, and I have consequently given some thought to the question—What can physiology contribute toward an understanding of the species problem?

The problem of species is a corollary of the theory of evolution. So long as we believed that living things were created each according to its kind, there was no species problem. The acceptance of the view that dissimilar modern forms have arisen from common ancestors through descent with modification cast the biologist into a dilemma not unlike that which confronts the modern physicist who must consider light now as a wave, and now as a corpuscle. How has the discrete, "corpuscular" character of the natural groups which we call species arisen from the "wave" of continuous change which underlies evolution?

Classical evolutionary theory is based primarily on morphological data. These data are formulated under the name of comparative anatomy; a mature body of doctrine in which the rules of the game are well established and agreed upon. We might expect the apparent sister science of comparative physiology to contribute something to evolutionary theory, but an examination of the facts is sadly disappointing. Comparative physiology is not only a younger sister to comparative anatomy, having not yet found time to collect much data—she is also a weak sister, having developed no principles of her own.

[1] Presented at a joint symposium on "The Species Problem" of the American Society of Zoologists and the American Genetics Society, Princeton University, December 30, 1935.

If one considers the development of function, as revealed in any chapter of comparative physiology, it appears that certain general principles are employed wherever the function is exhibited. The discovery of these principles is the chief interest of the general physiologist. Their nature depends upon the peculiarities of the physical and chemical mechanism of the phenomenon in question. As general principles they are necessarily immutable and they appear or disappear in evolutionary development only as the mechanisms which express them occur.

The development of function in any series of organisms is effected in two ways. The quantity and quality of the chemical substances making up the essential physico-chemical mechanisms may alter; and the general anatomical arrangements through which the function is performed may develop in a variety of ways.

To take the comparative physiology of vision as an example, we have as general principles the laws of light and dark adaptation, intensity discrimination and the visibility of the spectrum displayed in similar form by mollusks, arthropods, and vertebrates. These laws depend in form upon the general nature of the photochemical system which appears to be common to these groups of animals. Quantitatively these visual functions vary greatly in different animals, depending on the particular qualities and quantities of the photochemical substances present in each case. Color vision depends upon the presence of at least two photochemical substances with different absorption spectra. We believe that in man there are four such substances, and that one of them, visual purple, is localized in a morphologically distinct receptor cell—the rod. The specific peculiarities of these aspects of vision depend upon the development of variety in the chemical substances concerned in photo-reception.

Visual acuity and the formation of a retinal image depend in addition on the general morphological structure of the eye. The problem has been met in entirely dif-

ferent ways by the arthropods and vertebrates. In the vertebrates one finds a variety of minor modifications in the structure of the retina and in the optical mechanism which may be correlated with the peculiar habits of different groups. The organisms have developed no new physiological principles in bringing these functions to perfection. Rather they have devised new morphological arrangements to take advantage of certain general physical principles.

The nervous system provides perhaps the most successfully developed branch of comparative physiology. This appears to be due to the fact that the function of the nervous system depends primarily upon anatomical arrangements. The evolution of function in the nervous system is the development of greater structural complexity and of more centralized control through localized central connections. Accompanying this development little change has been detected in the nature of the fundamental processes of excitation and conduction. Thus comparative neurology must always deal largely with morphological description.

In so far as the development of function depends on morphological modifications, comparative physiology can not be separated from anatomy as a science with unique concepts. Perhaps this is why it has been to date relatively unproductive except as a system for cataloguing data.

One of the rules of the game in comparative anatomy states that analogous structures do not count. Analogies are physiological resemblances, and it results that much of comparative physiology is ruled out *a priori* from the game of tracing evolutionary relationship.

This limitation clearly applies to the part of the development of function which depends upon general anatomical arrangements. That part which depends upon alterations in the quantity and quality of the chemical substances which make up the essential physical-chemical mechanisms must be considered further. Before doing

so, however, I wish to make one additional point concerning comparative physiology in relation to evolution.

The study of physiology demonstrates that successful function depends on the nicest adjustment between the anatomy, physiology, and chemistry of the organism and the properties of its environment. These relations are not frequently obvious until physiological analysis is complete. Yet until they are comprehended, the treatment of "fitness" and "survival" must remain superficial.

The transition of vertebrates from an aqueous to a terrestrial habit required many changes of which those in the organs of locomotion and respiration are notable. The evolution of the pentadactyl appendage is one of the classical materials of comparative anatomy. Its treatment deals with the disposition of bone and the supporting musculature. The accompanying evolution of the neurological mechanism of posture and locomotion is commonly ignored—in fact was practically undiscovered at the time the principal anatomical factors were first set forth. We now know that a very elaborate neurological mechanism is concerned not only in the production of motion in the limb, but also in relating its posture to that of the body as a whole. The fact that the fish's fin is primarily an organ of equilibrium rather than of locomotion is of the utmost importance, for it shows that long before the problem of standing or running arose in the terrestrial vertebrate, the neurological mechanism for postural coordination was laid down in the paired appendage.

The physiology of respiration is particularly illuminating in connection with the problems of fitness and the environment, since it is concerned with the control of simple physical exchange between organism and environment. That terrestrial animals require a respiratory organ which, unlike the gills of fishes, is not injured by the dryness of the atmosphere, is obvious. That the lung imposes on the respiratory surface an environment essentially different from that of the air or water is easily

overlooked. The alveolar air must always contain a much higher concentration of carbon dioxide than the atmosphere. The effect of this is far-reaching. The blood of many fish, normally aerated by exposure to waters in equilibrium with the atmosphere, is quite unable to transport oxygen if exposed to carbon dioxide tensions, such as occur normally in the mammalian lung. The evolution of the respiratory mechanism in the terrestrial vertebrate has involved changes in the chemistry of hemoglobin and in the regulation of the acid-base balance which have paralleled the more obvious alterations in the respiratory organs.

These considerations serve to emphasize the multiplicity of the changes which must simultaneously occur in any successful evolution of function. Perhaps they have some bearing on the relative importance of small versus large mutations, concerning which I believe Professor East is to have something to say. The chief contribution of physiology as such to the species problem appears to me to lie in the more complete description which it affords of the nature of the organism and which enables us to analyze with more intelligence the phenomena of fitness, adaptation and survival.

Let us return to the chemical substances which take part in the evolution of function. Professor Gortner, in an essay published some years ago, discussed the relation of biochemistry to the problems of organic evolution.[2] His emphasis was on "the great harmony, from the biochemical standpoint, which exists in nature." Although he carefully avoided drawing the conclusion, the inference seemed intended that this harmony has arisen through evolution from common origins. Certainly few among you would question this view. Our interest at the moment is not in the similarities, but in the dissimilarities of a chemical nature which differentiate the various natural groups of organisms.

[2] R. A. Gortner, *Scientific Monthly*, 30: 416–426. 1930.

If our systems of classification have any reality, surely they should serve to indicate the relation of one set of aspects of the organic world as well as another. I have been entertained to consider what a chemist would make of the classification of animate nature—a naive chemist, blind to all structural relations of larger than molecular dimensions. Consider such a chemist, equipped with his modern techniques and supplied with neatly labeled specimens through the cooperation of his biological colleagues, much as they receive from him their carefully bottled C.P. reagents. Would he produce a classification similar to that which we employ?

After reviewing his material, he would undoubtedly set up two great kingdoms—the chlorophylophores and the chlorophylophorophages. The former he would distinguish by the universal presence of a certain magnesium-porphyrin-phytol complex with characteristic absorption spectrum. With this he would find associated the ability to perform a number of most important synthetic processes, including the construction of a diversity of carbohydrate and nitrogenous substances from simple inorganic substrates. The chlorophylophorophages hold all the rest. So far, so good, for our blind chemist has come pretty close to separating the animal and vegetable kingdoms.

Being, like most of his fellows, beholden to the medical profession, our biochemist would now turn his back on the chlorophyl-bearers. He would notice that the chlorophylophorophages fell into two sub-kingdoms—those which synthesize their own amino-acids, and those which lack the full power to do so. The aminoacidogens display many chemical properties in common with the chlorophyl-bearers, and the botanist would tell him that there were biological resemblances also. Doubt would arise as to whether this group really belonged among the chlorophylophorophages. Could they be degenerate forms which have lost their green coloring matter? No, the

chemical criterion is too definite, the functions served too fundamental to permit of doubt.

The sub-kingdom of organisms which lack the power to make their full complement of amino-acids from inorganic substrates would also trouble him, for into it would fall creatures as diverse as pathogenic bacteria and the laboratory rat. He would note that there was very little evidence for placing the many intervening members of this sub-kingdom there at all; and would admit that much more work needs to be done. On the whole, he would prefer that his students did not question him too closely about these divisions of his classification and would turn the discussion to the more satisfactory criteria employed to separate the various phyla.

Like other systematists, our chemist will select striking and easily discernible characteristics to separate his major groups. Because it lends itself to his methodics, he has given much attention to the fluids of the body. In one of these, the blood, he has discovered a group of colored substances which possess the common property of uniting with oxygen so loosely that they serve admirably the function of respiratory transport. In this these substances are unique and they are seized upon to characterize one of the principal phyla of the new classification—the Chromosanguinea.

This phylum is readily broken down further into classes and orders based on most definite properties of the respiratory proteins. The nature of the metallic element universally present serves to separate the classes—the Cuproproteinata, yielding copper proteins or hemocyanins, and the Ferroproteinata, in which iron is the most essential element concerned in the respiratory transport. The hemocyanins fall into two groups, based on the proportion of copper in the molecule and on certain general physico-chemical characteristics. One group provides an order into which the snails and the horseshoe crab are placed—the other takes care of the cephalopods and decapod Crustacea. Obviously the classification of

the older naturalists which grouped the snails with the cephalopods as mollusks and included *Limulus* with the Crustacea as arthropods was based on mere morphological resemblance.

The class Ferroproteinata would contain three definite orders. One of these is characterized by an iron-porphyrin-protein called chlorocruorin. The porphyrin in this molecule is distinct from that occurring in hemoglobin. To this order may be assigned the sabellid and serpulid worms, a satisfactorily compact group of organisms. The second order is characterized by an iron-protein complex which does not contain porphyrin. It is called hemerythrin and is produced by the siphunculid worms and by one member of the polychaet worms, a group which ordinarily contains hemoglobin. The third order is distinguished by the presence of hemoglobin in the blood and he will call it the Protoporphyrina, from the particular porphyrin which composes all hemoglobins.

The Protoporphyrina is in many ways a satisfactorily natural group. It contains all vertebrate animals, a group in which there is not only much morphological similarity, but in which on the whole the chemical characteristics display some uniformity. To it also must be assigned the majority of annelid worms, from which some at least have traced vertebrate ancestry from morphological considerations alone.

Unfortunately there is literally a fly in the ointment; the larval *Chironomus* contains hemoglobin in its blood stream, as does the snail *Planorbis* and a number of other mollusks. Clearly the anatomical resemblances which have caused *Planorbis* to be classed along with the other hemocyanin-bearers as a gastropod are merely "parallelisms" or "convergences" in development. Our chemist is convinced by this time that morphological arrangements, which you will remember were too large for him to apprehend and hence too vague for him to comprehend, have led the biologists to an entirely erroneous classification of animals and plants.

It is unnecessary to follow him further in his attempted classification. Rather let us inquire why he has been led to such foolish conclusions from the logical consideration of a very definite series of facts. First, you will note that he has built his system on a *single* series of characters. If he had taken all the known chemical facts into consideration, he might have arrived at conclusions more satisfactory to the biologist. For instance, the chemical composition of the organic and inorganic materials in the shell would place *Planorbis* among the snails in spite of the presence of hemoglobin in its blood, as would, no doubt, many other facts which might be learned about the chemistry of its tissues. This is not, however, the chief difficulty.

Our chemist has not stuck to his last. I have allowed him unwittingly to think as a biologist as well as a chemist. In setting up a phylum based on respiratory pigments, he has "lumped" according to physiological rather than strictly chemical criteria. He has failed to distinguish between the analogies and homologies in biochemical mechanisms, and as a result he has produced a classification as naive as that of Aristotle. It is well known that quite distinct chemical substances serve analogous functions in different groups of organisms. For example, phosphocreatine, which appears to play an indispensable rôle in the contraction of vertebrate muscle, is replaced by phosphoargenine in the muscles of many invertebrates. Hemocyanin, hemoglobin and hemerythrin serve a common function. They do so because they have certain physico-chemical properties in common; not because there is any generic relationship between the chemical configurations which happen to give them these properties. The resemblance between the respiratory pigments of the cephalopods and the vertebrates have no more evolutionary significance than do the similarities of the eyes in these groups. Both are remarkable cases of convergence to serve a common function.

If the distribution of chemical peculiarities among the natural groups of organisms is to be given an intelligent

interpretation, we must first develop some satisfactory criteria by which to judge what resemblances are significant in an evolutionary sense and what are not. We need some body of chemical doctrine, similar to that which embryology has given to the morphologist, by which to judge our findings. We must know, not only what substances occur here or there, but also how they come to be where they are, from what they are made and how their occurrence is determined.

The complex molecules which are of interest in this connection are not made up out of whole cloth by the organism. Natural syntheses do not differ from those of the laboratory in this, that the larger molecule is put together from smaller fragments or *vice versa*: protein from amino acids, polysaccharide from simple sugar, vitamin A by the disintegration of carotene, etc. A not unlimited number of basic configurations appear to be used repeatedly by animals and plants in forming complex molecules of the greatest importance. The sugars, fatty acids, amino-acids and their derivatives have long constituted the principal materials of biological chemistry. More recently the wide-spread occurrence of sterol, porphyrine and isoprene derivatives in a variety of physiologically active substances has attracted attention.

The various groups of organisms may be distinguished by their abilities to construct, transform or utilize these substances. A well-known English biochemist and geneticist has written:

> I cannot synthesize a bun
> By simply sitting in the sun;
> * * * * * * * *
> But readers, rhyzostomes and rats
> Are fairly good at making fats.
> So let us concentrate on this.
> Our most effective synthesis.[3]

This lament emphasizes that the evolution of what we are pleased to consider a higher organism is accompanied by a specialization in certain biochemical capacities.

[3] J. B. S. H., *Brighter Biochemistry*, December, 1924.

The interpretation of the chemical data concerning organisms can only be made in terms of the natural history of the syntheses which produce the substances of interest. A consideration of some facts concerning hemoglobin emphasizes this point. Hemoglobin occurs in all vertebrates and in many worms, thus correlating well with the natural classification. But it also occurs sporadically in a few mollusks, insects and other forms which have in general solved their respiratory problems by other means. Hemoglobin is a compound of iron, protoporphyrin and protein. The specific differences in the hemoglobins from various animals are attributed to differences in the protein, since the porphyrin is the same in all. Some latitude is permissible then in the composition of the protein which goes to make a hemoglobin. Protoporphyrin, combined with iron as hematin, is known to occur as a constituent of a number of substances, such as cytochrome and catalase, which are wide-spread throughout the animal and vegetable kingdoms. Since these substances seem to serve very generalized metabolic processes, it is clear that almost all organisms must possess the ability either to synthesize or acquire and use hematin. While I can not pretend to understand how a snail comes to undertake a new chemical synthesis, still, these being the facts, I find it easier to believe that *Planorbis* has gotten its hemoglobin by combining the hematin it already possessed with some protein it already could make, than to consider hemoglobin in all its complexity arising *de novo*. The distribution of complex substances is intelligible only in terms of the distribution of the precursors from which they are formed.

Comparative physiology and biological chemistry alike can contribute to an understanding of organic evolution only in so far as they observe the old rule of the game—the distinction between analogies and homologies. Does this mean that they have nothing more to contribute than new facts to be brought in line? I think not. We all have a presentiment that the substance determines the

form, not the form the substance. We know already of a number of substances, hormones, which through effects on the growth of specific tissues profoundly alter the form of the organism. Many specific characters are based on pigmentation, which is nothing else but a direct chemical product. Some of you are thinking of genes as bodies of molecular dimensions, or as complexes in which the organization does not differ greatly from that of molecular aggregates. Genetics and experimental morphology alike may be expected to utilize chemistry as profitably as have other branches of physiology. To do so, they will need to know not only what the active substances concerned in their phenomena are, but how they arise, multiply and undergo variation.

It has always been a mystery to me how the chemical potentialities of the adult are carried through the egg to the next generation. The problem is an old one in so far as morphological potentialities are concerned. In the case of the simpler chemical phenomena, it would seem more susceptible to solution. Does the egg carry the seed, as specific enzymes, perhaps, of all the substances the adult is to form? And how do these seeds multiply themselves during growth? This is also the problem of the gene, though you know at least that the chromosomes are present in the egg. The recent isolation of a crystalline protein possessing the properties of tobacco mosaic virus by Dr. Stanley gives some hope that we may learn how this multiplication is performed.[4] When a little of this substance is introduced into the tissues of a healthy tobacco plant, the material is reproduced in quantity. It is true that the cooperation of living tissue is, as yet, required for this multiplication. This condition will, however, be with us so long as we are considering the problems of genetics and development. The important point is that the foreign substance and the normal tissue together cause the production of more of the specific

[4] W. M. Stanley, *Science*, 81: 644. 1935.

foreign substance. This discovery promises to bear fruit in fields far removed from the tobacco patch.

Finally I wish to call your attention to certain limitations which the chemical nature of organisms place on the nature of variation. This matter has been considered recently by Pantin.[5] The chemical materials of living bodies are each entities, characterized by specific atomic composition and arrangement. The more complex molecules are, it is true, combined from numerous simpler units, as in the case of the proteins, and some variation and gradation in their properties is obtained by combining these units in different ways. Even these complex molecules can only be made of certain kinds of unit and modified by changing whole units at a time. Frequently, as in the case of the hemoglobins, successful function is dependent on the presence of a particular unit in the larger complex. It results that the organism can never be infinitely plastic. Its variation is limited by the variation of its chemical substance. It must occur step by step, each step as discrete as those which separate the species which chemists designate as different compounds.

I conclude that physiology and chemistry can contribute to an understanding of the development and relationships of modern organisms only if they observe the distinction between homologies and analogies. Much of physiology is by nature analogous—being the fortuitous combination of factors to serve a complex end. The morphological factors are the province of comparative anatomy; the chemical factors deserve treatment by a similar discipline. Before they can aid us in understanding the evolutionary problem, we must develop criteria for judging their true homologies. This will come as we learn more of the origins of the substances we find in particular organisms and understand something of the mechanisms underlying their multiplication and variation. When it comes our studies will be on a new footing.

[5] C. F. A. Pantin, Linnean Society's *Journal-Zoology*, 37: 705–711. 1932.

INTERSPECIFIC STERILITY[1]

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So complicated is the species problem that certain aspects regarded as cardinal points by some are not even mentioned by others. Sterility which ensues from species crosses is one of these aspects. Bateson never failed to point to interspecific sterility as a central problem. "Until this event"—the origin of a sterile hybrid from fertile parents of common origin—"is witnessed, our knowledge of evolution is incomplete in a vital respect" (Bateson, 1922). On the other side some recent writers dealing with evolution do not include interspecific sterility among their topics.

Definitions of the term "species" are manifold, but most of them contain a reference to the fact that the crossing of two forms classified as belonging to different species leads to sterility. Sterility as a result of crossing has even been made *the* criterion of speciation, although nearly all descriptions of taxonomic species have been given without any test as to sterility in crosses with other taxonomic species. But in most cases when an attempt has been made to cross two such taxonomic species, sterility has been encountered. How does this come about? The taxonomist, if he is careful, bases his judgment on the study of a large group of individuals collected over a large area. He then finds that in spite of the variability in the morphological characteristics of his form, a definite gap exists which separates it from other groups, even though these other groups might be found in the same area. He then reasons that this gap would not exist if intercrossing between the two forms would be frequent and would give fertile offspring. This reasoning might

[1] Presented at a joint symposium on "The Species Problem" of the American Society of Zoologists and the American Genetics Society, Princeton University, December 30, 1935.

be based on obsolete ideas of blending inheritance or on Mendelian inheritance in multiple factor cases. In both cases it leads to the same result, the assumption of interspecific sterility as intrinsic in the definition of species. This definition becomes strained on different occasions under the weight of organic manifoldness. Only one of these shall be mentioned here. There are cases in which the acute eye of the taxonomist fails to distinguish morphological differences, while the breeding test reveals the existence of groups sterile in intercrossings. Have we to rely on this test and elevate these groups to "physiological species" or have we to follow the morphologist and call them at best "biological races"? An answer to this question will depend on a consideration of interspecific sterility in its different aspects. Not having given a final definition of the category "species," our discussion will not be restricted to the undoubted cases only.

Three different questions might be asked as to our problem: (1) What is the phenomenon? (2) What is its genetic basis? (3) What is its evolutionary origin?

THE PHENOMENON

Interspecific sterility is a term which covers many different facts. One extreme is represented by the inability or the unwillingness of two species to permit a union of their gametes. Near another extreme we encounter the appearance of hybrids vigorous somatically, but with varying degrees of failure to produce fertile gametes.

Only a few remarks may be made in regard to the failure of gametes of different species to accomplish fertilization. In many animals there is a lack of attraction of the sexes from different species which acts as a barrier to gametic union. In other cases copulation occurs, but no positive reaction between egg and spermatozoon ensues. Similar failure is frequent in many examples of external fertilization.

Little is known about the interaction between the soma of the female of one species and the spermatozoa of the

other species. But it is probable that the physiological and possibly the mechanical conditions within the female ducts might not always be favorable to motility and functioning of the foreign spermatozoa. A similar type of interaction is of great importance in the determination of sterility or fertility among higher plants. Success in interspecific crosses depends to a large extent on the interaction between the female sporophyte tissue of the pistil and the male gametophyte, the pollen. As in many higher animals, however, a positive interaction between the diploid female tissue and the genes does not insure fertilization. The pollen tubes, having grown through the style, may not be able to fuse with the ova.

A survey of the fate of hybrid zygotes, obtained from crosses in which the barrier to fertilization was absent or not insurmountable, reveals another wide array of facts. Most disturbances, and we are concerned only with cases of incomplete compatibility, occur only in one of two phases of the hybrids' life cycle, namely, at the beginning of development or at the time of sexual maturity. While the disturbances in the embryonic stage, in which as a second embryonic stage might be included the period of pupation in insects, lead generally to an early death of the zygote, a lack of serious developmental disorders on the other hand generally allows for the growth of a strong hybrid. The more astonishing, therefore, at first sight appears the failure to produce offspring. We are inclined to think first of this aspect of hybrid sterility, and the impression is often gathered, that its visible cause is a failure of normal chromosome conjugation during the meiotic prophases. To be sure, many cases of hybrid sterility are closely bound up with these processes and the explanation, known for many such cases, is so convincing that their weight becomes easily disproportionate. However, disturbances of chromosome pairing per se do not necessarily bring about sterility. Other processes, particularly in animals, are often responsible for such sterility.

It is important in this connection to remember that the infertility of hybrids has two principally different aspects. A hybrid might be sterile because it does not form functioning gones,[2] but it also might appear sterile because its gones, though functioning, give rise to inviable zygotes ("Parasterility," Brieger, 1930). In such cases crosses are sometimes possible in which the gones of a "sterile" hybrid of this kind can function and give viable offspring. Many of these latter cases, examples of which will be given later, are connected with failure of normal chromosome conjugation.

Here we shall discuss the inability to form functioning gones. It frequently originates away from the meiotic processes, both before and after their occurrence. Thus in the female hybrids between *Drosophila melanogaster* and *D. simulans,* degeneration of oogonial tissue regularly sets in before synapsis and the deposition of yolk would be expected and these two processes do not occur at all (Bonnier, 1924). In the male hybrids from the same cross degeneration likewise occurs early, the whole testis being rudimentary. Development of spermatogonia into spermatocytes rarely begins (Shen, 1932). Very similar conditions were found in certain moth hybrids in the genus *Pygaera* (Federley, 1931).

Detailed information is available for the spermatogenesis of hybrids between the two lines A and B ("biological races") of *Drosophila pseudoobscura* (Dobzhansky, 1934). According to the type of cross, the general structure of the testis is rather normal or it is abnormal. In both cases alike, however, abnormalities in the finer cytological details occur. Partly, these relate to chromosome pairing, but the decisive factor obviously lies in gross abnormalities of the first maturation spindle and subsequent cell-division. No second meiotic division occurs and only abnormal spermatids are formed. Less

[2] A gone (Renner) is a cell (and its direct descendants) resulting from the reduction division. This term covers both the gametes and the gametophytes.

striking but perhaps not less important abnormalities occur already in the spermatogonial stages.

Among plants the same two phases in the hybrids' lives which were mentioned before are critical stages, namely, the early stages, involving the formation of the embryo and the late stages of attaining to sexual maturity. These late stages have been subdivided into the differentiation period of the various flowering organs, the differentiation period of the archespores and that of the gones developing from them. Considering only the late stages, we might quote that certain derived hybrids between the willows, *Salix caprea* and *S. viminalis,* fail to form inflorescences but produce vegetative shoots instead (Heribert-Nilsson, 1918), or that interspecific hybrids in the genus *Epilobium* often fail to form flowers (Akerman, 1921). A very complete series of disturbances appearing in various stages of the formation of the ovaries and pollen sacs have been described in different hybrids between *Nicotiana* species (Brieger, 1928). To mention female sterility only, plants were found in which the gynoecium had never formed a placenta, others in which development had halted at an early placental stage, still others in which well-formed placentae had either failed to form ovules completely or where ovule-formation had been abnormal or had not progressed beyond early stages. All these examples relate to stages prior to chromosome conjugation and meiotic divisions.

Sterility phenomena in phases past the maturation divisions are of two different kinds. The first type occurs in plants only in which the gones have to undergo a certain degree of development in order to form the gametophytes. Here the genetic constitution of the gone itself may produce degeneration. The other type occurs both in plants and animals. It is due to an unfavorable interaction between the gones and the parental tissue. On account of the higher dependence of the gametophytes of higher plants on the sporophyte, this type is frequent among plants and often affects the female and male gones

differently, due to the different degrees of dependence of the two kinds of gametophytes. In animals, sterility due to degeneration of gametes after the meiotic processes is very rare. An interesting case, although not strictly belonging in the category of *interspecific* sterility, will be mentioned here, and referred to again later. This is the sterility of *Drosophila melanogaster* males which lack one or both of the "fertility-factors" in the Y-chromosome (Shen, 1931). Here spermatogenesis is normal in all respects and leads to the formation of apparently normal spermatozoa. However, the spermatozoa, having entered a certain part of the testis, fail to become motile and finally degenerate. It is probable that the cause for this late breakdown in the process does not lie in the germ cells themselves but in a deficiency of the somatic tissue of the testis.

What, we may ask in conclusion of this section, is the general phenomenon of hybrid sterility? It is the inability of many hybrid zygotes to develop normally. Abnormal development in organs necessary for the individual's life leads to early death. This accounts for the critical embryonic stage of hybrid development (including to some extent gones of hybrid origin), for here organs are formed and begin to function. If none of these organs are seriously handicapped in their formation, the hybrid will grow up to full size and function. But there is one more task to fulfil, the production of functioning gametes in animals or of flowers and spores in higher plants. Failure in these stages does not affect the individual, due to the high degree of independence of soma and germinal cells; but it results in sterility. Lack of production of viable offspring and sterility of hybrids in interspecific crosses are thus essentially similar phenomena.

THE GENETIC BASIS OF INTERSPECIFIC STERILITY

To say that interspecific sterility results from an inharmonious genetic constitution is only a restatement of the

facts. It leads to a certain analysis when we inquire as to the nature of the genetic differences between the species involved. Are all kinds of genetic categories involved—plasmatic differences, gene-mutations and chromosomal changes? Are they of equal importance? How many differences are present?

Our knowledge is scanty. There is high probability that the plasma or, more accurately stated, extranuclear elements, are concerned with interspecific sterility in some plants. But reciprocal differences in the success of crosses or in the hybrids' sterility in other organisms may be based on nuclear factors which impress the plasma with a maternal effect. This has been established for the *Drosophila pseudoobscura* hybrids (Dobzhansky, 1935a; Dobzhansky and Sturtevant, 1935).[3]

There are some well-established examples in which the term "disharmonious gene combinations" in interspecific hybrids implies a fuller understanding. It was found that in crosses between the two species of hawk weeds, *Crepis capillaris* and *C. tectorum,* the results were either viable hybrid offspring or inviable embryos (Hollingshead, 1930). This is due to the presence of a certain gene in *C. tectorum* in two allelic forms l and L, both of which are in harmony with the genom of their own species, but one of which brings about a lethal development in the presence of other genes introduced by hybridization. A similar situation which, though giving viable combinations, is in extreme cases responsible for hybrid sterility in the cyprinoid fishes *Xiphophorus* and *Platypoecilus* (Häussler, 1928; Kosswig, 1929, 1931; Gordon, 1931). Crosses between different races of the two species are successful and produce fertile hybrids. However, if a

[3] The question is undecided in lepidopteran species crosses. Here the observed reciprocal differences are either based on plasmatic differences between the species or on differences between the Y-chromosomes which normally are transmitted together with the cytoplasm from mother to daughter (Federley, 1929, 1932; Bytinski-Salz, 1930). Goldschmidt's demonstration (1934) in Lymantria that the plasma and not the Y-chromosome is concerned with intersexuality points to the plasma in other cases too.

black race of *Platypoecilus,* distinguished from other races by a single factor difference, is used in the interspecific cross, hybrids originate in which melanotic tumors appear. The genetic analysis shows that *Xiphophorus* contributes a number of "modifiers," the rôle of which in their original genom is unknown; in the hybrid the modifiers cause a disharmonious action of the *Platypoecilus* color gene. Other hereditary cancer-like growths in interspecific crosses which can lead to sterility are known in the genus *Nicotiana* (Kostoff, 1935).

Although the foregoing cases have been presented as examples of interspecific sterility due to gene mutations, this is not to be taken too literally. While one factor could be isolated as a differential deciding between sterility or fertility, its action only becomes decisive in the general genetic background of the hybrid. How far this involves differences of the order of gene mutations and of chromosomal changes and how many such differences are present are still open questions. The fact that more than one genetic difference is concerned becomes especially distinct in a factorial scheme. Such a scheme has to make use of complementary factors present in the two species not producing sterility by themselves but in combination. One species then would contain AA bb, the other aa BB, resulting in a hybrid Aa Bb, which would be sterile. This represents a minimum formula and it could be extended to include as many factors as necessary. We see then that one single genic change does not differentiate a new cross-sterile line from the old one.

Are chromosomal changes by themselves responsible for interspecific sterility? We can classify such changes into two groups, (a) quantitative changes—duplications and deficiencies taken in the widest sense and (b) rearrangements—inversions, translocations, possibly fusions and fragmentations.

That gross chromosomal differences of all possible kinds are very frequent between different related species is, of course, well known. To judge the rôle of these dif-

ferences in individual cases as to interspecific sterility is difficult because so many kinds of differences are generally existent between two species that an evaluation of a single difference becomes impossible. We shall therefore try to estimate the rôle of chromosomal differences from a theoretical consideration of different types.

We shall begin with the quantitative changes. There is one type of such changes probably occurring in plants only about whose rôle in evolution we are perhaps better informed than about any other. This is the increase of whole chromosome sets after hybridization which leads to allopolyploids. The allopolyploids are not only morphologically distinct from but also often sterile with their parents, though fertile among themselves. They thus possess specific rank. The cause of the interspecific sterility is at least partly independent of the original hybrid nature, as it is often observed between autopolyploids and the original diploid. It has been shown in some cases that the interaction between the male gametophyte and the female sporophyte is disturbed. When fertilization between a tetraploid and a diploid is successful and the triploid hybrid is viable, then the maturation divisions cause sterility of almost all gones. For the gones will contain new quantitative changes in their chromosomal material. Duplications of some chromosomes, deficiencies of others, and in addition, in case of crossing-over between non-homologous hybrid pairs, duplications and deficiencies for chromosome sections, will cause an unbalance of the genetic constitution which in most cases will be fatal for the gametophytes.

If we consider the rôle of duplications and deficiencies in interspecific sterility in general, we might inquire if the presence in relation to the original species of one duplication or one deficiency may lead to sterility. Zygotes deficient in homozygous condition for genes present in the original species are not viable as a rule. By the production of deficiencies a new homozygous form is thus not obtainable in general. In the rare cases where such forms

are viable and fertile the hybrid with the original form, being only heterozygous for the deficiency, will *a fortiori* be viable and fertile also. The same holds true for crosses between an original form and a new one possessing a duplication in homozygous condition. Such derived forms are more frequently viable and fertile, but the hybrid again will be viable and fertile also. In summary: simple quantitative genetic changes, apart from some special cases in plants, do not lead by themselves to interspecific sterility.

We shall now consider chromosomal rearrangements in respect to interspecific sterility. A single inversion by itself should lead to sterility in certain cases only. A zygote obtaining homologous chromosomes from both parents with only one sequence of genes changed will be viable and not show abnormalities in the general processes of production of gones. However, pairing may be interfered with between the two chromosomes heterozygous for the inversion. Disjunction will probably lead to a majority of normal gones in females, but not in males in cases where crossing over occurs in this sex (Beadle and Sturtevant, 1935). A certain amount of unbalanced gones will then be formed which will either not function at all or give rise to unbalanced zygotes. Such unbalance, as we have seen in our discussion of quantitative changes, leads often to inviability of the zygotes. Single inversions could thus provide a basis for a certain degree of hybrid-sterility between two fully fertile strains, each homozygous for one or the other gene arrangement. Single inversions in more than one chromosome pair would accentuate the condition. Numerous inversions within different chromosome pairs might disturb pairing so considerably that unbalanced gones become the rule in both sexes and lead to considerable sterility of the hybrid gones.

Translocations between autosomes produce similar results (Fig. 1). Two parents, one of which is homozygous for a single autosomal interchange, will produce viable

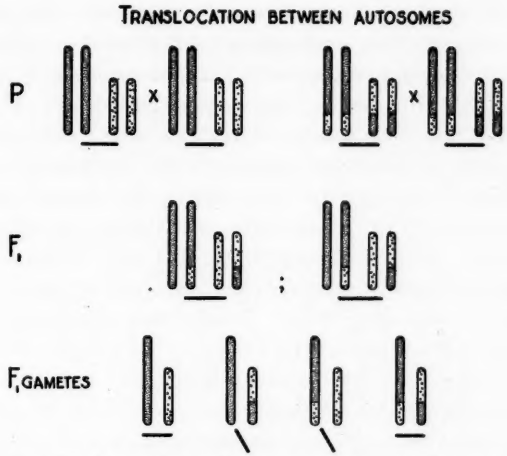

FIG. 1. Reciprocal translocation between two pairs of autosomes. P. Normal (left) and translocation (right) females and males. F_1: Hybrids between the two parental lines. The horizontal lines indicate a balanced genic condition; the slanted lines denote genic unbalance (60° slant is used when homozygous deficiencies are present, 30° when the unbalance is based on the presence of heterozygous deficiencies or of duplications only (see later fig.)).

zygotes with two full genoms. However, in the hybrid semisterility, the production of 50 per cent. (in some cases less) unbalanced gones will ensue. Accumulation of auto-

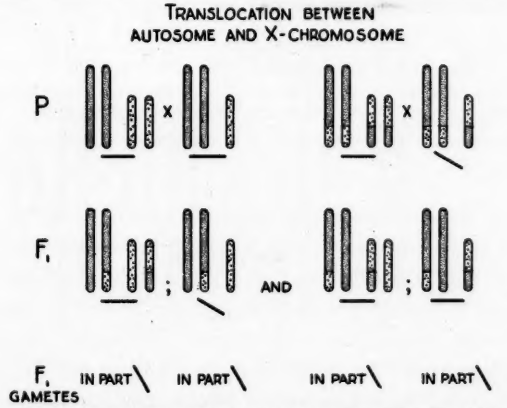

FIG. 2. For explanation *cf.* Fig. 1 and text. The offspring of both reciprocal crosses of the P-lines is given.

somal translocations between more than two pairs of chromosomes will again increase sterility. Five such interchanges, for instance, will allow only for 3 per cent. fertile gones in maize (Brink and Burnham, 1929).

Interesting conditions can result when translocations involve the sex-chromosomes. A line homozygous for a reciprocal translocation between an autosome and an X-chromosome (Fig. 2) can not be obtained in a balanced condition, as the heterogametic sex will contain both a duplication of part of the X-chromosome and a deficiency of part of the autosome. This would make the heterogametic zygotes generally inviable. In case of viability of the translocation line the F_1 zygotes of both sexes would be completely balanced after a cross in one direction, while the reciprocal cross would yield balanced zygotes of the homogametic, but not of the heterogametic sex. All F_1 individuals, however, would produce in part unbalanced gametes. A reciprocal translocation between an autosome and a Y-chromosome (Fig. 3) could lead to a stable line, if the Y-chromosome is completely "empty." The presence of two Y-chromosomes in both sexes, each

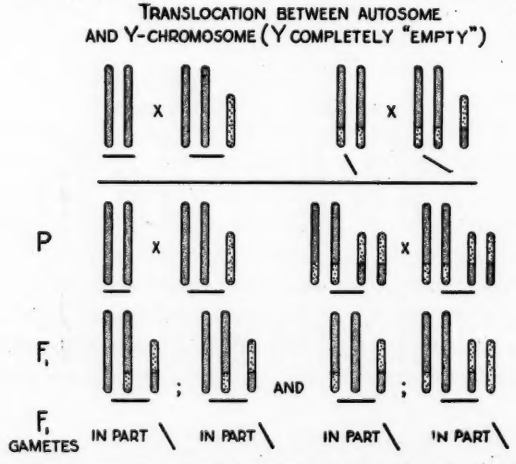

FIG. 3. Upper part: Translocation without increasing the number of Y-chromosomes in both sexes results in unbalanced zygotes. Below: Results of crosses between two balanced lines.

reciprocally interchanged with an autosome, would be necessary (such a line would appear to belong to an XO-type of sex chromosome). The cross between such a derived line and the original one would give viable offspring which, however, would produce in part unbalanced gones. Finally, reciprocal translocations between X- and Y-chromosomes (Fig. 4) would not permit the establishment of a constant line.

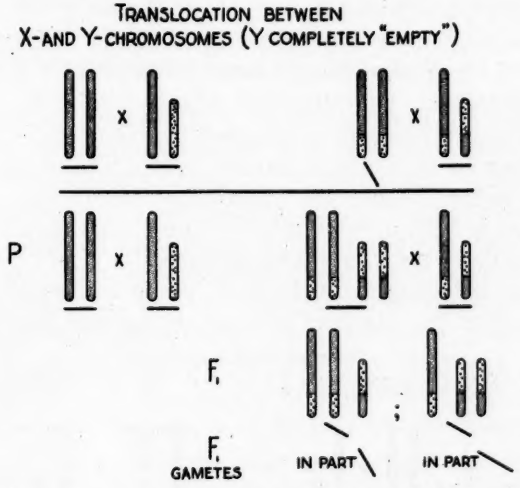

FIG. 4. Upper part: Translocation without increasing the number of Y-chromosomes results in unbalanced homogametic zygotes. Below (right): Addition of two translocation Y-chromosomes to the homogametic sex provides for genic balance. However, the offspring of the translocation parents has new, unbalanced constitutions, thus not permitting the establishment of a true breeding stock.

We have thus far considered cases in which genetic material was exchanged by both chromosomes involved in the translocation. If only one chromosome furnishes the genetically active material, be it through non-reciprocal simple translocation or, more probably, through exchange involving inert chromosome material of one chromosome, the results would not be changed fundamentally in case of translocation between autosomes or between

autosomes and the X-chromosome. Neither would there be a change in the cases involving the Y-chromosome, as our former assumption of a completely "empty" Y-chromosome classifies these cases already into the present group "transfer of inert material." The Y-chromosome, however, is not completely empty.[4] Besides containing in certain organisms genic material of the usual kind in the genus *Drosophila,* and perhaps in other forms, it is also concerned with the fertility of the male sex. In *D. melanogaster* it is known that at least two different sections of the Y-chromosome have to be present to insure male fertility. How this will affect sterility in crosses between two lines, distinguished by translocations, will be shown by an example, involving transfer of one "fertility section" onto the X-chromosome (Stern, 1929). Crosses in both directions between a normal line and one homozygous for the translocation will give fertile F_1 females but completely sterile F_1 males will result in one cross (Fig. 5). Matings between the normal line and the

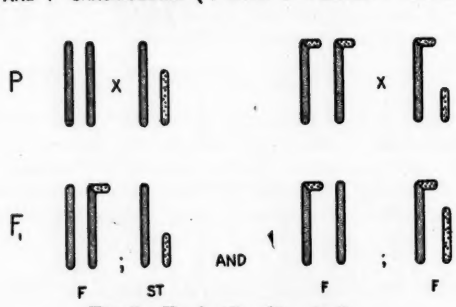

FIG. 5. F = fertile; St = sterile.

translocation line, into which attached X-chromosomes have been introduced, will give fertile F_1 females again, while completely sterile F_1 males will result from both possible crosses (Fig. 6).

[4] If it were it would have been lost in evolutionary history through nondisjunction.

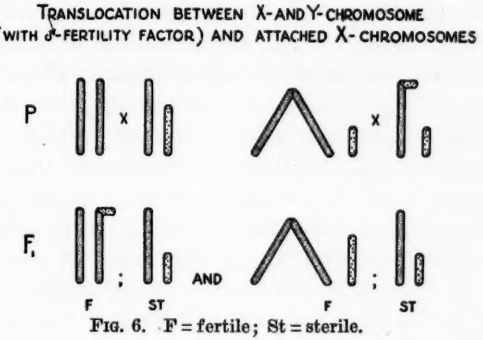

FIG. 6. F = fertile; St = sterile.

If we summarize the effect of chromosomal rearrangements on sterility, we find that conditions can occur in which two homozygous, fully fertile lines produce offspring with reduced fertility. But this partial sterility affects only the gones derived from the hybrids or the F_2 zygotes. No case is found in which the F_1 zygote itself is of reduced viability resulting in embryonic disturbances or zygotic abnormalities in the formation of gones. Only the Y-chromosome translocation involving the fertility factors results in complete zygotic sterility of one sex. It is possible to go one step further (Kozhevnikov, 1935).

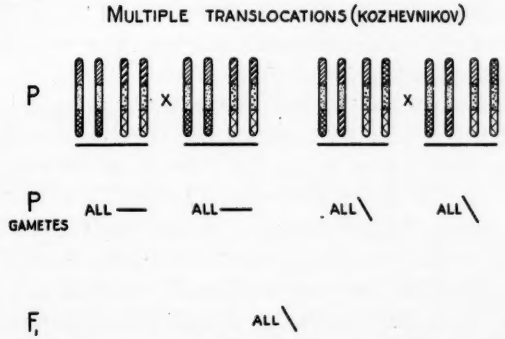

FIG. 7. P, left: Normal race with chromosomes 2 and 3, three different sections in each pair are indicated. P, right: translocation race containing one normal chromosome 2, one chromosome consisting of middle section 2 and two upper sections 3; one with middle and lower section 3 and upper section 2; and one with middle and lower section 3 and lower section 2.

A race of *Drosophila melanogaster* has been "constructed" recently, which differs from the normal line by a number of complicated translocations (Fig. 7). It is constant but produces almost exclusively unbalanced gametes. This brings about nearly complete cross-sterility with standard stocks, as all unbalanced zygotes fail to develop. Here, then, we have a case closely resembling natural interspecific sterility and based purely on chromosomal rearrangements. Unfortunately, however, and this is an intrinsic feature, the artificial line is itself in a kind of balanced lethal stage, giving only 50 per cent. viable zygotes.

What is the bearing of our findings concerning chromosomal changes on interspecific sterility? It seems clear that no single chromosomal change, either of a quantitative nature or a rearrangement, can produce the result typical for probably the majority of cases of interspecific sterility, namely, the production of inviable or *zygotically* sterile hybrids from homozygous fertile parents. The one exception, the Y-chromosome fertility translocation, can at best account for certain cases only. And in the form presented in the diagram, which is derived from actual experiments, no successful maintenance of the translocation stock would occur in nature, as the free fragment of the Y-chromosome would be soon eliminated by the sterility of the hybrid males, while the unfragmented Y-chromosome would survive.

Furthermore, if we examine the results of some of the most pertinent studies on the differences between nearly related species which give sterile hybrids, our conclusions are strengthened. *Drosophila melanogaster* and *D. simulans* have chromosomes whose general structures are strikingly alike, chromosome for chromosome, section for section, with only occasional small deviations (Pätau, 1935). One large inversion is present (Sturtevant and Plunkett, 1926) which by itself is not responsible for the weakness of the hybrids, whose disharmonious development ranges from non-viability of zygotes to viable but

completely sterile individuals with degeneration of the germ cells before meiosis. The two "biological races" A and B of *Drosophila pseudoobscura* again are much alike in their chromosomal architecture, although 6 inversions and differences in size and shape of the Y-chromosomes are present (Dobzhansky, 1935; Tau, 1935). The male-sterility, as described in an earlier section of this review, is independent of the pairing abnormalities between the hybrid chromosome groups and it can be demonstrated that the Y-chromosomes play at best a very subordinate rôle, if any, in this hybrid sterility.

If single genic changes can not bring about differentiation of zygotically intersterile lines, if single and even multiple chromosomal changes fail to cause such differentiation, what, then, is the genetic basis of interspecific sterility? Clearly it is a *complex* differentiation, founded on the presence of many genic differences. These genic differences most probably include changes of genes, quantitative differences in genic content and mutational changes connected with chromosomal rearrangements (position effects). The bringing together in the hybrid of the two genoms differentiated in many ways results in a genetically unbalanced zygote. To conclude thus that the disharmonious development of hybrids is due to unbalanced genetic content seems a result hardly worthy its name. But it is the only general statement of the genetic basis of interspecific sterility which our analysis has left.

The Evolutionary Origin of Interspecific Sterility

It follows from our conclusions regarding the genetic basis of interspecific sterility that its evolutionary origin occurs in more than a single step. Only a combination of genic differences makes its bearers cross-sterile with the rest of the population. This combination itself can, of course, conceivably be attained in one single recombinational process. But the sudden origin of a homozygous form cross-sterile with a different homozygous form of

equal descent is excluded. Interspecific sterility originates not differently in evolution from other multifactorial attributes of organisms and must therefore be subject to the same evolutionary laws, but it acts in a particular way. The accumulation of genic differences, if carried to a certain point, brings with itself the production of unbalanced hybrid zygotes. Once this singular point in the evolutionary process is reached, the isolation effect helps further the development of divergence from the rest of the population. Interspecific sterility thus is no prerequisite for divergent evolution. Whatever degree of isolation is needed, its initial character is more likely to be of a nature of spatial or temporal isolation in the widest sense. Such isolation could conceivably bring about differences which a taxonomist might regard as of specific rank. And it happens that very often differences based on complex genetic alterations are not compatible with hybrid development. But this, then, is a by-product, not a basis, of evolution. This conclusion is derived not only from our genetic analysis but agrees also with the demands of mathematical deductions. "In too small a population there is nearly complete fixation, little variation, little effect of selection and thus a static condition modified occasionally by chance fixation of new mutations leading inevitably to degeneration and extinction" (Wright, 1931). Cross-sterility at an early evolutionary stage would involve too small a population cut off from the parental population and thus stifle evolution instead of furthering it.

The study of partial isolation whose rôle has been measured by theoretical considerations (*cf.* Wright, 1931) is an important approach, at present but hardly begun, to the understanding of specific evolutionary processes. It may demonstrate environmental factors and genetic causes. But the primary genetic basis will hardly be of such a nature as to make the normal development of hybrid zygotes impossible.

To sum up: The phenomenon of interspecific sterility is the inability of hybrid zygotes to develop normally. Abnormal development results in early death of the zygote or degeneration of reproductive cells at various stages. The genetic cause of abnormal development is a complex difference of parental genoms leading to unbalanced hybrid zygotes. The evolutionary origin of interspecific sterility lies not at the beginning of divergent evolution but occurs in the course of it as a by-product.

LITERATURE CITED

Bateson, W.
1922. *Science*, 55: 55–61.
Beadle and Sturtevant
1935. *Proc. Nat. Acad. Sci.*, 21: 384–390.
Bonnier, G.
1924. *Acta Zool.*, 5: 1–122.
Brieger, F.
1928. *Planta:* 315–362.
1930. "Selbststerilität und Kreuzungsterilität." Berlin, 395 pp.
Brink, R. A., and Burnham, C. R.
1929. Am. Nat., 63: 301–316.
Bytinski-Salz, Hans, and Günther, A.
1930. *Zeits. induktive Abst. u. Vererbgsl.*, 53: 154–234.
Dobzhansky, Th.
1933. *Proc. Nat. Acad. Sci.*, 19: 950–953.
1934. *Zeits. Zellf.*, 21: 169–223.
1935a. *Proc. Nat. Acad. Sci.*, 21: 443–446.
1935b. *Genetics*, 20: 366–376.
Dobzhansky, Th., and Sturtevant, A. H.
1935. *Proc. Nat. Acad. Sci.*, 21: 566–570.
Federley, Harry
1929. *Hereditas*, 12: 271–293.
1931. *Zeits. Zellf.* 12: 772–816.
1932. *Bulletins of the Laboratory of Genetics, Acad. Sci. U. S. S. R.*, 9: 121–128.
Goldschmidt, Richard
1934. *Zeits. induktive Abst. u. Vererbsl.*, 67: 1–40.
Gordon, M.
1931. *Am. Jour. of Cancer*, 15: 1495–1519.
Häussler, G.
1928. *Klin. Wochenschrift*, 7: 1561–1562.
Hollingshead, Lillian
1930. *Genetics*, 15: 114–140.
Kosswig, Curt
1929. *Zeits induktive Abstgs. u. Vererbgsl.*, 52: 114–120.
1931. *Zeits. induktive Abstgs. u. Vererbgsl.*, 59: 61–76.

Kostoff, D.
1935. *Genetica*, 17: 367–376.
Kozhevnikov, B. Th.
1935. AM. NAT., 69: 459–461.
Pätau, Klaus
1935. *Naturwissenschaften*, 23: 537–543.
Renner, O.
1929. "Artbastarde bei Pflanzen." Hdbch. d. Vererbungsw. II, A. 161 pp.
Shen, Tun Hui
1932. *Zeits. Zellf.*, 15: 547–580.
Stern, Curt
1929. *Zeits. induktive Abstgs. Vererbgsl.*, 51: 253–353.
Sturtevant, H. A., and Plunkett, C. R.
1926. *Biol. Bull.*, 50: 56–60.
Tan, C. C.
1935. *Genetics*, 20: 392–402.
Wright, Sewall
1931. *Genetics*, 16: 97–159.

GENETIC ASPECTS OF CERTAIN PROBLEMS OF EVOLUTION[1]

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THERE is a general feeling among specialists in other departments of biology that the geneticist has made no contributions toward the solution of the more general problems of species origin. These biologists are evolutionists—but merely because the wealth of circumstantial evidence presented by Darwin makes the notion plausible, and because all facts discovered later are consistent with the idea. The whole concept remains for them, in form and factual content, just as outlined by the author of the "Origin of Species," with perhaps more precise information on the subject of variation, with somewhat more exact knowledge of the laws of inheritance and with certain changes in the values allotted to such factors as acquirements, mimicry, hybridization, isolation, and the like. Few would grant that ideas as to the methodology of evolution are substantially clearer now than they were seventy-five years ago.

Such a position is not consistent with the facts. The most important single obstacle in the path of Darwin's rationalization of the evolution process was the current reliance on the principle of blending inheritance. During the first third of the present century, this impediment was disintegrated and swept away. The establishment of the particulate theory of inheritance, with units of heredity arranged in linear fashion in the chromosomes and distributed by definite laws, has made it possible to calculate precisely what must happen to a given population under any particular system of breeding, when the type of chromosome mechanism existing in the population is known. And the chromosome mechanisms have

[1] Presented at a joint symposium on "The Species Problem," of the American Society of Zoologists and the American Genetics Society, Princeton University, December 30, 1935.

been typed for some thousands of groups. It only remains, then, to calculate the end results of the process or processes involved when appropriate values are given to mutation rates, to survival values and to other pertinent factors. Many concrete problems of this nature have been solved by the activity of such men as Wright, Fisher and Haldane; and not a few of the formal mathematical solutions coming out of the mill have been checked satisfactorily by experiment. Unfortunately, this work is sealed to many on account of the intricate mathematical processes involved; but one should not forget its existence or deny its significance merely because he will not take the trouble to learn the language in which it is written. The facts are that we possess to-day a body of ascertained knowledge about heredity so great and so well organized that its implications solve for us something like half of the problems of evolutionary methodology that puzzled Darwin; and we should be proud of it. The details have not been fitted together in many cases; but the general philosophy of the situation has been made quite clear.

On the other hand, some new difficulties have arisen in connection with the significance of the variations which are the raw material for evolution. Here, I think, some just criticism lies against geneticists for being too much concerned, perhaps, with specific theorems of inheritance and not sufficiently concerned with the bearing of these theorems on evolution. It is to this particular phase of the subject that I wish to draw attention to-day.

Variations are the primary phenomena of organic evolution. It is convenient to group them into (1) gene changes and (2) chromatin rearrangements.

Naturally, there remains an assemblage of subjects having connotations full of evolutionary significance, such as reproductive value, population pressure, species competition, migration, isolation, and so on; but it is rather obvious that these are all secondary questions having to do with selection pressure, which are not hard

to evaluate formally under the system of heredity known to prevail. Each presents certain practical difficulties in deciding upon rational values for them consistent with the facts and in choosing the mathematical manipulations necessary in reckoning their effects; and this spade work will furnish employment for some time to come. In the philosophical sense, however, the situation is so clear that the business of the details becomes a chore.

In addition, one is still confronted with problems having to do with the effective causes of variations. But less is being said of mystical forces—entelechies, and the like—and more plans are being made to put known physical agencies to test. Interesting results have been obtained by the use of x-rays and radium, the application of heat and cold and the submission of organisms to other environmental variations. Whether the changes induced by such means are wholly comparable with the changes found under natural conditions is not certain; yet no one conversant with recent work in genetics denies that they are similar in type. At all events we may rest assured that variations are produced either by definite agencies of this general character—in which case we shall ultimately find out something about them; or that they are the result of properties inherent in protoplasm, as pictured by Driesch, Bergson and others of their class—in which case we shall probably learn no more about them than we know at present.

Let us reverse the order of the two classes of primary evolutionary phenomena, discussing chromatin rearrangement first and gene changes second.

Three simple rearrangements of chromatin are known where the genic constitution remains the same in both quantity and quality. Only the structure is different.

The first is merely an inversion of a block of genes. At first sight, one is inclined to see no reason for supposing that variation is caused by such a shift. There is some evidence, however, that the architecture of the germ-plasm is not wholly without importance. It is quite

possible that there is a "position effect," that genes in the sequence ABCDEFG are not necessarily the same, physiologically, as genes ABEDCFG. Just how much importance such shifts may have in descent is another matter. If the inverted gene block is relatively large, gene-by-gene chromosome pairing with a normal mate is hampered, and sterility is increased. It is difficult, therefore, for a race homozygous for the inverted block to become established. If the inversion consists of only one or two genes, perhaps pairing will be normal, but one is hardly warranted in attributing much evolutionary significance to a process involving even a tendency toward disrupting an established mechanism. On the other hand, the most striking difference between *Drosophila simulans* and *Drosophila melanogaster* is an apparent inversion. Whether this change is the effective cause of the differentiation of the two forms is unknown; nevertheless, a case where an inversion has become established should not be overlooked. Perhaps the tendency of normal and inverted to be infertile together is a physiological isolation factor of some importance after the two biotypes have originated.

The second is simple translocation, where a block of genes changes its party affiliations from one chromosome to another. In its extreme form, this process could provide for a reduction in chromosome number. The latter is not known experimentally, though there are numerous instances where it may have been involved—cases such as the genus Crepis, in which progressive reduction of chromosome number is thought to be indicated by the circumstantial evidence. It is difficult to see how this action could further species differentiation.

The third rearrangement without gain or loss of chromatin is reciprocal translocation. This phenomenon, first suggested by Belling, has made it possible for Renner, Cleland and others to make a complete and wholly satisfactory analysis of the relationships of the species in the genus Oenothera. The story has passed into genetic his-

tory and need not be repeated here. It is sufficient to point out that reciprocal translocation is essentially a means of reducing the number of independent chromatin carriers and also of the amount of interchange within the carrier. In other words, the greater the amount of ring formation in Oenothera, the less the number of chromosome recombinations possible and the smaller the amount of crossing-over. One suspects, therefore, that differentiation of the genus Oenothera has been hindered rather than helped by the introduction of the process, though stability may have been promoted.

The loss or gain of existing chromatin may now be examined.

It is hard to imagine how the loss of chromatin could have been an important means of advancing organic evolution. A given number of genes assorted into a definite number of chromosomes appears to be characteristic of each species of organism. Cases are known where individuals lacking chromosome pieces, or even whole chromosomes, do not seem to be handicapped in a serious manner. But it must be realized that these individuals possess one complete genom plus something less than a second complete genom. For this reason, development may be normal. Selective elimination of descendants occurs, however, because gametes $N-x$, even when functional, seldom undergo development when union occurs with others of the same constitution. Occasionally it may be that $2N-2x$ individuals exhibit normal life cycles; nevertheless, there is always a suspicion that such instances are examples of previous duplication of the missing parts. Evidence brought forward by Bridges and Demerec on Drosophila indicates that genic deficiency is lethal when homozygous, even if only a single gene is missing. There are a few apparent exceptions where deficiency in a small portion of the germ-plasm is not completely destructive to all cells ("cut" and "yellow" are possible examples), but it is conceivable that results here are conditioned by earlier reduplication, as indicated by successive groups of similar bands in the salivary glands.

Redundancy of chromatin is not so disorganizing as deficiency. Numerous examples are known in datura, maize and Drosophila where the genetic constitution is 2N+x. These individuals often differ from the normal type to a degree that competition with the latter is imaginable. But it is hardly to be supposed that the fixation of such abnormalities is important as an evolutionary process. In the first place, balanced forms of the type 2N+2 usually stand closer to the wild type than the unbalanced forms of the constitution 2N+1. In the second place, relatively few wild species are known where such an origin is indicated by the chromosome constitution of near relatives.

The addition of complete genoms is of more consequence, but only among plants. Artificial production of haploids through induced parthenogenesis or of tetraploids and higher polyploids by preventing pairing at meiosis through raising or lowering the temperature, ordinarily results in forms exhibiting little deviation from the normal. Haploids may be slightly smaller, and tetraploids slightly larger—or at least slightly stockier—than the diploids; but there is no significant indication of qualitative differentiation. Moreover, frequent emergence of autopolyploids under ordinary conditions of reproduction is restricted by the irregularities of gamete formation set up by the union of more than two chromosomes at meiosis. Thus an autotetraploid may be formed at once by the prevention of pairing, and a triploid may be obtained by crossing this tetraploid with the diploid; but chromosome distribution is so disorganized that even the forms with even numbers of chromosomes rarely persist, unless a change occurs permitting bivalent pairing. Polyploidy is supposed to be a matter of more importance, therefore, when it follows the hybridization of forms previously differentiated from each other to a degree that will allow bivalent pairing immediately. It is assumed that the numerous polyploid series found in the flowering plants have been formed in this way. No

other means appears to be available consistent with our cytological knowledge, since chromosome doubling through fractionation is prevented by the inability of chromosome parts lacking spindle fibers to produce them Further, allotetraploidy has been duplicated and new species created by experimental means. At the same time, it should be realized that the union of natural species followed by amphidiploidy is a rare phenomenon.

It thus becomes exceedingly questionable whether any of these aberrant cytological processes are important in the evolutionary sense. Allopolyploidy can produce a new species in a single step, but only as a special type of end result following hybridization. Hybridization, that is to say recombination of variant genes, has undoubtedly been a primary factor in the evolution of both plants and animals; but polyploidy has been valuable only in the higher plants, presumably because hermaphroditic reproduction has prevailed there. It would hardly be defensible to give high rank to a process of evolution unavailable to most animals.

Another source of change has been suggested by investigators studying the large salivary chromosomes of the Diptera. It is a speculation based wholly on the fact that repeated band patterns are frequently found. The conjecture is made that the original life unit was a gene capable of multiplying into free genes of like character, thus increasing the opportunity for gene mutation. As time passed, the capacity for orientation into gene-strings arose; and gene reduplication end-on-end may be assumed to have been retained as long as asexual reproduction persisted. With the rise of sexual reproduction, however, pairing difficulties would arise, except where reduplication takes place in the earlier ancestors of the germ cells.

Such imaginative pictures are no more than mental setting-up exercises at present; but while we are going through them, may I be permitted a word along the same line. The so-called inert region of the chromosome, situ-

ated in the spindle fiber region, may be a chemical laboratory where occasionally a new gene is organized without materially disturbing the chromosome architecture previously built.

Since these chromatin rearrangements which lie outside the ordinary reassortments of genes typical of sexual reproduction prove to be evolutionary agents having little significance, one must fall back on gene changes. Gene mutation is unquestionably a phenomenon of unparalleled importance in organic evolution. Here the material is obtained for novel patterns. A careful scrutiny of gene changes and gene activity, therefore, ought to give us a clearer impression of how phylogenetic differentiation has come about. Unhappily geneticists have been somewhat negligent in dealing with this task. They have been too much concerned with gene arrangement in the chromosomes and gene reassortment at gametogenesis and fertilization. This concentration solved the riddle of heredity as completely as the establishment of the modern atomic theory solved the riddle of chemical recombination—and in the same sense; but it also threw out of focus the broader evolutionary problems that are functionally related.

Perhaps I may be allowed to state categorically some of the things we know or infer about the gene without being required to confirm them. Confirmation could be made, given sufficient time. Genes can not be greater than 100 or so of the larger proteid molecules. They are more likely to be single molecules of this order of magnitude, for they behave as individual molecules rather than as structures. They possess a power of reproduction that is essentially perfect. They may change or mutate, and the fact that such mutations are frequently reversible indicates that the changes are a form of isomerism analogous to chemical isomerism. Unit deficiency, when homozygous, is usually lethal, even to single cells; which has led to the inference that every gene is indispensable to the organism. But it is fairer, perhaps, to argue that

genes vary greatly in the importance of their activities during ontogeny and that various specimens might be dispensed with were it not that this would cause disharmony in the machine as a whole. The activity of each gene is effective on numerous characters; and each character is the end effect of the operations guided by many genes. Genes begin their special activity[2] at different periods of development, and may take active parts over periods of varying length. At all events, it has been shown that in numerous instances physiological processes are taken to a given stage of completion by a given combination of genes, whereupon a new combination takes charge and pushes the process further, to a point where still another set takes command. Thus one may think of a developmental process as a serial affair much like the building of a motor car, with specialist genes directing each step.

On this basis of fact, one may proceed to picture the nature of the gene mutations required if they are to persist under the stringent selection of natural agencies.

The two organisms whose germ-plasm architecture is best known are *Drosophila melanogaster* and *Zea Mays*. Approximately 1,000 mutant genes are known in the one and 400 mutant genes in the other. Developmental physiology, however, is best known in man; this is true, at least, of post-natal development. Now the mutations investigated in the first two species nearly all tend to be recessive to the wild type or so-called normal type. Moreover, a large proportion of them are lethal in the homozygous condition; and the great majority of those which are viable are less viable than the wild type, if they are concerned with operations having physiological importance. In superficial characteristics—for example, most colors of organs—viability may not be affected. Nevertheless, even in these instances one gets the idea that the wild type gene, the gene that has survived natural selection, is able to work harmoniously with other

[2] Genes presumably may be exhibiting some activity at all times.

genes and to produce constructive, positive reactions; while the genic mutants, on the other hand, work less harmoniously with the other genes of the constitution and are less positive and constructive in their processes. And this view is confirmed when one examines the cases where a given locus is haploid through deficiency. If the haploid gene is wild type, the organism is apparently unaffected; but if the haploid gene is a mutant, its presence is almost invariably detrimental. Bar-eye, an interpolated gene having no normal mate, is a good example of the second phenomenon. At the same time, it should be noted that in man—as also in poultry—while there are numerous mutations known which are recessive to normal, there are many genes producing abnormalities of skeleton, eye and other organ systems that tend to be dominant to the normal.

The situation is so peculiar that taxonomists have little interest in the characters with which geneticists deal, maintaining that they are wholly unnatural material for evolutionary processes. Professor C. T. Brues has examined the published descriptions of mutational effects in Drosophila, at my request, and finds that only a limited few characteristics of similar type have ever survived in nature, and these often in distant genera, thus indicating that the germinal causes are not identical. It is worthy of note, however, that the characters resembling the Drosophila mutations that have survived elsewhere in the insects are invariably retrogressive simplifications.

The tendency of the Morgan school, in the face of this situation, is to remain silent. When the subject is mentioned, the reader is led to infer that only a very meager percentage of chance mutations should be expected to be of evolutionary importance. R. A. Fisher, however, is not inclined to dismiss the matter so cavalierly. He accepts the rate of detected mutations in Drosophila as a fair approach to the true rate—as apparently the Drosophila investigators also do—yet since calculations based on alternative inheritance demand higher mutation

pressures, he has developed a theory of the evolution of dominance by the selection of subsidiary genes, to meet the point.

In my opinion, neither of these attitudes is satisfactory. Higher mutation rates than those observed in Drosophila *are* required, if it is not assumed that evolution has now virtually stopped. And mutations of a different character than those ordinarily observed are essential, unless some such theory as that of Fisher is accepted. Now Fisher's postulates have been subjected to certain destructive criticisms by Wright and by Haldane; and with these criticisms I am in sympathy. But there is another, and more devastating, reason why dominance can not be evolved from recessiveness except in very special cases. It accords with the evidence to suppose that each gene has qualities of its own. If, therefore, we think of characters in terms of gene activity in series, it is obvious that physiologically defective genes, which include virtually all the described Drosophila mutations, can not be made physiologically constructive by the selection of other genes. The most one can say is that, through epistasis, the actual result in certain cases may resemble superficially what is demanded by the Fisher theory.

The very fact that we are impaled on the horns of such a dilemma surely means that something pertinent has been overlooked. To find it, we must divest our minds of the nonsense which has come to surround the words *dominant* and *recessive,* and do a little plain thinking about the relation between genes and characters.

Since any given character is the result of the activities of numerous genes working both concurrently and serially, these activities must be harmonious if the organism concerned is to be favored in the struggle for existence. Three different effects of mutations are therefore conceivable: (a) the machinery may be stopped entirely because the new gene can not do essential work previously performed by the old gene, and the result is ordinarily a lethal; (b) the machinery may be restricted in operation,

giving a physiologically less complete end product; (c) the machinery may be diverted from its original manufacturing tendencies into the production of an end product different from the original.

Clearly, the first two groups should be recessive to the normal, since it is known that a single normal genom is sufficient to carry on the processes of ontogeny. Judging from their behavior, most of the mutations detected in Drosophila and Zea belong to these types, and can not be expected to be important in evolution unless the evolution be retrogressive. The third group should be an admixture of several sub-groups. Under certain conditions it may be supposed that during the development of a new end product cytoplasmic activities will be so changed that the normal genom (G) can not function in its usual manner if in combination with the new genom (G′). Thus there will be an apparent dominance of the mutant over the wild type brought about in a manner quite different from that usually imagined. I suspect that many of the dominant abnormalities found in man and in poultry are of this nature. One can not more easily imagine an evolution founded in the main upon disharmony of this nature than upon the physiological restrictions characteristic of our first two groups. This being true, one is forced to predicate progressive evolution solely upon gene mutations fitting harmoniously into the series of activities of the genes remaining unchanged. Gene changes having evolutionary significance, therefore, are likely to give end products varying but slightly from those already evolved, either qualitatively or quantitatively. The new genom, when combined with the old, may sometimes show an approach to dominance or an approach to recessiveness; but it is clear that the effect of the new gene must be such as will retain, for the series of reactions in which it is involved, a perfection comparable to that of the existent wild type. For this reason, it may be assumed that in a hybrid of this nature, G and G′ will react together, each responsible for a complete activity of its own, thus pro-

ducing a hybrid pattern in which dominance of the classical type is not emphasized.

Now what evidence is there for the existence of frequent mutations of this nature? They must be frequent. Evolution under the particulate inheritance mechanism demands higher mutation rates than those found for the detected changes in Drosophila and Zea; and if the majority of these are thrown overboard, we are left somewhat empty-handed.

I suggest that constructive mutations *are numerous,* but have ordinarily remained unnoticed simply because destructive mutations are more easily described, catalogued and scored, and therefore have been more convenient in genetic research. There is evidence of a varied nature, nevertheless, in support of the idea that constructive mutations occur with remarkably high frequency.

I have presented elsewhere a study of a large series of species hybrids in the genus Nicotiana where normal genoms developed under natural selections are pitted against other and different genoms developed under natural selection. There the effect of innumerable gene differences of the constructive type may be examined; and where the effects concern essential characteristics, dominance is practically absent. Development of the alien genoms together gives a harmonious result in which the normal activity of each is traceable in the resultant pattern of every organ.

Evidence as to the precise moment of origin of mutations of this class is not so definite as one could wish, and experimental proof of the exact location of the genes concerned is meager indeed; but this is to be expected from the very nature of the phenomenon. Evidence of their abundance is not lacking. Every plant breeder has noted that no matter how similar different varieties appear to be, there are literally hundreds of gene differences affecting the quantitative relationships of every organ. Easily detected recessives may be hard to find as material for work; but inherited quantitative differences, giving all

the results expected under multiple factor control, are omnipresent. Moreover, I have various records of diploid Nicotianas originating presumably from induced parthenogenesis—and theoretically complete homozygotes—whose immediate progeny were as much alike as if cut out by a die, but whose later descendants, coming from successively selfed mother plants, rapidly became more variable in every part. This increase in variability could only come about from frequent mutations of the type we are discussing.

Plant breeders, as I have said, may be expected to receive this view with sympathy, for their experience will corroborate it, whether they are dealing with wild plants or with cultivated plants among the Angiosperms or with lower forms. At least, so I read the evidence presented by experimental taxonomists, by agronomists and horticulturists, and by experimental pathologists, such as Stakman. But how about zoologists? I read their results the same way when they are dealing with naturally selected forms, which will cross together and sometimes exhibit fertility—Sumner on Peromyscus, Phillips and others on birds, the fish hybridists, and even the interesting series of mutations described by Raffel in Paramecium.

From long experience in trying to fix details of structure after crossing—using as a standard of comparison either plants resulting from induced parthenogenesis or plants produced by immediate asexual division—I have the feeling that existent gene variation of this character is 5 to 10 times that of the destructive type. This does not necessarily mean, however, that there is the same relative difference in mutation frequency, for the constructive mutations should usually survive, while the destructive mutations should often be rapidly eliminated.

If this hypothesis is sound, it gives us a different picture of certain genetic results. Hitherto, if red-eye Drosophila mutated to white-eye, or if vermilion-eye reverted to red-eye, or if agouti in mammals mutated to albino, the result was always supposed to be the same thing. Yet

there is no means, except superficial resemblances, for judging that the phenomena are identical. Most of the color changes used by geneticists are presumably of slight physiological importance. They may be either destructive or constructive, in the physiological sense. All one can say is that the red eye-white eye locus is at a given point in the Drosophila X, and agouti is at a given point in a particular chromosome of the mouse or the rabbit or the guinea pig. There is no reason why one should not suppose that there are numerous different ways by which agouti may mutate to albino. There is no reason for not assuming that agouti may mutate frequently and still produce the general effect from which the name comes. In fact, nearly every fence corner in the West is inhabited by a family of Peromyscus that is slightly different from the one in the next fence corner, not only in coat color but in almost every characteristic. I am emphasizing this point because evidence is accumulating daily that there are hosts of multiple allelomorphs in our genetic material. If they are easily scored, we give them different names; if they are difficult to separate, we lump them. All of which is unobjectionable as a practical matter, but becomes objectionable if we do not recognize the theoretical implications involved. Those who have worked with self-sterile plants, where the phenomenon permits the recognition of allelomorphs not otherwise detectable, know that the number of allelomorphs which can be isolated is only limited by the extent of the patience of the investigator. It is within the bounds of reason, therefore, to assume that the development of the proper micrometric methods is all that prevents the detection of hundreds of allelomorphs at other loci that are now unrecognized. And these allelomorphs, one may venture to predict, will frequently be constructive in character.

Lack of time prevents further elaboration of these arguments. I must close, therefore, with a summary statement. It is this. The establishment of the particulate theory of heredity permits the computation of

rational results from any system of breeding under any assignable mutation pressure and subject to all conceivable selection pressures. Formal solution of the more pressing evolutionary problems presented to the consideration of biologists from Darwin's time onward is possible, therefore, provided stress is laid almost exclusively upon constructive gene mutations fitting the preexisting patterns of gene activity. Raw material of this type exists in quantity. It meets the requirements of physiology. It satisfies the demands of taxonomy. And it places orthogenesis on a sensible basis of chemical theory.

NOTE.—This paper was written in Ocober, 1935. I had previously (*Genetics,* 20: 403–451, 1935) discussed "constructive" and "destructive" mutations in a somewhat similar manner. On November 19, 1935, I received a letter from Dr. J. Clausen, in which he pointed out that E. Baur (*Bibl. Gen.,* 4: 144–147, 1924) and T. Tammes (*Z. I. A. V.,* 36: 417–426, 1925) had presented comparable views. Still later, Professor R. Goldschmidt wrote that both Sturtevant (*Car. Inst. Pub.,* 301, 1921) and he (he could not remember the citation) had considered the matter. Though I have been unable to discover any mention of the matter in this particular paper of Sturtevant, it is quite possible that it was treated at some other time.

Professor R. A. Fisher had also written to say that he has not considered the Drosophila mutations as a fair sample of mutations in general; that he does not feel that the evolution of dominance is an important matter in evolution; that he agrees with much that I had to say in my earlier paper on the subject, and that I have misunderstood his position. After rereading Fisher's papers on dominance, I am still unable to harmonize his published statements and his letter; nevertheless, I offer him my apologies for any unwitting misrepresentation of his views. I hope that he will make his present position clear at an early date.—E. M. E.

THE PHYLOGENY OF THE ADRENAL GLAND[1]

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INTRODUCTION

BEFORE discussing the mammalian adrenal on which this paper is based, I find it necessary to give a brief recapitulation of our knowledge of the adrenal in the lower Vertebrates. No adrenal homologues are found in Amphioxus nor is there any comparable structure in the Ascidians, although these animals possess in the neural gland a structure which is really a rudimentary pituitary gland.

With regard to Invertebrates, Gaskell (1912) and Poll (1906) have found bodies which they consider the equivalent of the adrenal medulla in the body cavity of the leech *Hirudo medicinalis,* but no similar bodies have been found in any other Invertebrates.

In the next Vertebrate group we come to the Cyclostomata—the pro-fishes, or lampreys, and of these, Giacomini (1902, 1912) has described two series of bodies in Petromyzon, one series irregular in shape, which are attached to the walls of the posterior cardinal veins, the renal arteries and the arteries dorsal in position to the kidney. These are the representatives of the cortex—the cortical or inter-renal bodies.

The second series are in the form of strands of tissue running along the large arteries and extending practically the whole length of the body cavity. These are the representatives of the chromaffin tissues of the higher forms.

In Bdellostoma so far only the chromaffin tissue has been found, the cortical tissue is no doubt present, but is

[1] Read to the Zoology Section of the Australian and New Zealand Association for the Advancement of Science, Melbourne, January, 1935.

more difficult to obtain than the former, which is easily demonstrated by placing a wad of cotton wool soaked in potassium bichromate for twenty-four hours in the body cavity, and then washing away the excess bichromate. The chromaffin tissues are stained brown and are easily seen on subsequent dissection.

Balfour (1878) described the chromaffin bodies in the sharks and rays as being situated on either side of the dorsal aorta, segmentally arranged. They show definite relations with the sympathetic ganglia and are homologous with the adrenal medulla. The cortical or interrenal substance, which is in the form of a single large body in the lower portion of the body cavity between the kidneys in sharks and dogfishes, is a paired structure in the same region in the rays. The bony fishes or Teleosts possess a number of cortical bodies associated with the ventral surface of the kidney—these are the corpuscles of Stannius; Giacomini found on the pronephros of eels has described the chromaffin tissue in the walls of the carcles of Stannius.

Giacomini discovered the chromaffin bodies in the Teleost fishes and found them to be groups of cells associated with the cardinal veins.

In the Ganoid fishes Stannius has found the cortical bodies scattered in the kidney substance, and Giacomini has described the chromaffin tissue in the walls of the cardinal veins.

The adrenal representatives of the Dipnoi (lung fishes) have not been completely worked out to date. In Protopterus, bundles of cells are scattered around the kidney, which are of doubtful nature. Giacomini found chromaffin tissue arranged in a segmental fashion round the intercostal arteries. No definite cortical tissue has been discovered so far in the lung fishes, a most anomalous position, for we find in practically all other Vertebrates the cortical tissue is essential for life.

The Amphibian adrenal takes the form of golden yellow strands of cells associated with the ventral surface of

the kidney. Microscopically these bands show a dual structure. The major portion of the glandular material is made up of branching and anastomosing cell columns, with masses of cells occupying the interstices, or situated on the ends of the ramifying cell columns. The former structure (cell columns) represent the cortex of higher adrenals and the latter represent the medullary (chromaffinic) elements of the higher animals.

In the Amniota, we find the reptilian and avian adrenal with the medullary cells lying in a network of cortical cells, the whole forming a compound body separate from the kidney.

Individual members of the group vary; for example, the present author has found the adrenal tissue in the boa constrictor to be represented by a series of colored, radially arranged, streaks of cells along the lobes of the attenuated kidneys.

Mammals alone among the Vertebrates possess a true cortex, but the adrenal in the Monotremes is not mammalian in structure, a varying amount of interlacement of the cortex and medulla taking place. In a specimen of Echidna examined by the author, cortex and medulla may be distinguished by their typical arrangement at one end of the gland, and at the other they interlace.

A constant reminder of the dual origin of the mammalian adrenal body is given by the presence of accessory adrenal tissue scattered through the body cavity of the majority of mammals. Sometimes accessory glands are found. Harley (1858) found four complete adrenals in a sheep; and the present author has found three additional adrenal bodies with cortex and medulla and zoned cortices in a specimen of native cat—*Dasyurus viverrinus*.

In the majority of mammals, the adrenals are associated more or less intimately with the antero-mesial borders of the corresponding kidneys. In addition to the main adrenals, there are two other bodies, the carotid bodies and the coccygeal body—both of which give a

chromaffin reaction. Proceeding caudally from the carotid bodies, we find chromaffin bodies attached to the superior and inferior cervical sympathetic ganglia and with the stellate ganglia, and most of the sympathetic ganglia caudal to the stellate possess small groups of chromaffinic tissue associated with them.

There is a large abdominal chromaffin body between the caudal regions of the kidneys and five or six subsidiary ones situated near it.

There is usually a fairly large accessory cortical body associated with each testis in the male.

Before discussing the nature of the adrenal in mammals I will give a brief description of the structure of the ideal adrenal.

It is a roundish elongated body composed of an inner medulla (chromaffinic tissue) and an outer investing cortex. At one region the cortex does not surround the gland, which comes to the surface at this point—the hilum. It is here that the adrenal vein usually leaves the gland.

The adrenal is covered by a capsule of varying thickness, which may, or may not, send trabeculae of fibrous tissue into the interior of the gland. It is composed of chiefly fibrous tissue, and may include varying amounts of unstriped muscle; and striated muscle fibers may also be present.

The outer region of the cortex constitutes the zona glomerulosa of Arnold (1866) and is usually composed of columns of columnar cells, which, near the capsule, bend through 180° and proceed back in their original direction. The main body of the cortex is usually made up of larger, rather more squarely shaped cells which tend to be arranged in parallel columns (fascicles). This is the zona fasciculata of Arnold (1866). The cells of this zone may contain varying quantities of lipoid droplets, pigment granules, etc. Centripetally these columns tend to branch and anastomose with one another, building up a reticular zone—the zona reticularis of Arnold.

In this zone the greatest aggregations of pigment frequently occur.

The medulla is composed of cells of varying shape, some square and medium sized, others large and spherical. The medullary cells are gathered together into columns or bundles, usually surrounded by connective tissue and there are numerous spaces or sinuses between the cell bundles.

The cells of the medulla may be shown by the bichromate method or osmic vapor method of Cramer to contain numerous granules of adrenalin. By the technique described by the present author both cortical and medullary cells may be shown, in the normal adrenal, to be packed with granules of vitamin C aggregated chiefly round the nucleus.

The Mammalian Adrenal

The adrenal glands of 250 species of mammals have now been described. Of these I have described, in as yet unpublished work, the adrenals of 133 species (comprising more than 400 specimens). Kolmer (1918) has described 67. Osman Hill (1930, 1933) and others are responsible for the descriptions of the remaining fifty species.

The Monotremes

The Echidna adrenal has been described by Elliott and Tuckett (1906), Kolmer (1918), MacKenzie and Owen (1919), of the Australian Institute of Anatomy, Basir (1931) and myself during the present year.

My results closely agree with those of MacKenzie and Owen and vary in parts in interpretation from those of Basir.

The Echidna adrenal does not exhibit a definite mammalian structure. Some adrenals have been found which show a mammalian disposition of cortex and medulla at one end and a reptilian arrangement at the other, that is, an interlacement of the cortical and medullary elements. Very large areas of the gland may, however, consist en-

tirely of cortical material and the medulla may be reduced to a small amount of tissue at one end. There is not a definite arrangement of the cortex into the zona glomerulosa, zona fasciculata and zona reticularis of Arnold (1866), but in some regions of the gland small areas exhibit a tendency towards the differentiation characteristic of these areas. In the case of the Platypus, however, the cortical cells seem aggregated around a series of veins which run in a longitudinal fashion through the glands, between these lobules, the cells are medullary in nature. At the caudal pole of each gland, as in Echidna, the medullary and cortical cells tend to form groups surrounded by connective tissue. There are no signs of areas in the gland which show indications of differentiation into the typical mammalian zones, so that in this respect the Platypus is lower than the Echidna.

In both these forms the glands are typically associated with the antero-mesial borders of the corresponding kidneys.

It appears that the adrenal of the monotremes is still in a state of flux. No definite plan is followed in the adrenal of either the Echidna or the Platypus.

The glands occupy the typical mammalian association with the kidneys, such a relation not yet being upset by any attempt of the animal to take an upright position either by climbing or attempting a bipedal mode of progression.

In view of the advance of the brain and adrenal of the Echidna over that of the Platypus it would appear that the development of land life by the Echidna has had a definite advantage over that of the aqueous environment of the Platypus.

It appears that one of the many factors which led to the persistence of this aberrant evolutionary stage in the animals of to-day was an insufficiently organized adrenal, such an array of abortive attempts on the part of an animal to form a typical mammalian adrenal is not found in any other mammal and it is of interest that all other mam-

mals above the Prototheria possess the ability to carry out uterine gestation. Modern outlook on the adrenal is that it plays a definite part in pregnancy changes and thus the efficient organization of the adrenal would be an important factor in the change from oviparity to viviparity, and this efficient organization is not present in the adrenals of the Monotremata.

The Adrenal in the Dasyurinae

The left adrenals in the three species of Dasyurus examined show minor variations in position. In *Dasyurus maculosa,* the gland is turned so that its broad side may be seen ventrally. In *D. viverrinus* the gland is situated about midway between the posterior vena cava and the left kidney and is not as elongated, while in *Dasyurus geoffroyi* the left adrenal is turned so that its thin edge is visible ventrally. The right adrenals of all three species are attached intimately to the posterior vena cava, which opens directly into the hilum of the gland.

In *Sarcophilus ursinus* there is a slight association with the posterior vena cava, but in the specimen examined a direct discharge from the hilum of the gland into the vena cava could not be demonstrated. The adrenals of the Dasyurids and Sarcophilus all showed a fairly voluminous medulla with some large sinuses and a rather thin rim of cortex, which, in the four species, showed definite fasciculation, the three Dasyurus adrenals all show definite zoning, but this is indefinite in Sarcophilis.

The Adrenal in the Phascogalinae

Species of Phascogale, Sminthopsis, Troughton's new genus Planigale, Dasyuroides and Antechonomys were examined.

The right adrenal in the genus Phascogale varies in position, but the general tendency is for attachment with the right vena cava, with or without additional attachments to the right liver or kidney. In *Phascogale mimulus* the right gland is definitely attached mesially to the

right kidney, exhibiting no other attachments, some specimens of *Phascogale flavipes* show attachment chiefly to the liver, but the vena cava is usually involved also. Dasycercus, a similar form, from which, it could be assumed from other reasons, the climbing and other Phascogales have probably descended, shows quite a variety of right adrenal attachments. The group of Phascogales with their varied types of right adrenal attachment provide an excellent starting point for the differentiation of the various types of right adrenal attachment observed in the other Marsupial groups.

The Phascogales also all show adrenals with a thin band of cortex surrounding a rather voluminous medulla.

The Cortex shows fasciculation in all of them, and in most, the zona reticularis and the zona glomerulosa are difficult to distinguish. Only in the case of one specimen (*Phascogale macdonnellensis*) was the zona reticularis observed to show to any extent. In this specimen, which was carrying a young one in the pouch, the zona reticularis occupied one third of the cortex. All the specimens of Sminthopsis examined show attachment of the right gland to the vena cava, in one or two cases the right liver lobe or right kidney were involved.

In all species but *Sminthopsis crassicaudata,* the cortex was thin; in this species it was much broader. All glands, young and old, showed a definite fasciculation of the large middle zone.

The Phascogales show a right adrenal attached to the vena cava. The glands possess a fasciculated cortex, with the zoning varying from indefinite to definite.

The main characters of the sub-family Phascogalinae are attachment of the right adrenal to the vena cava to a varying extent, a thin rim of cortex surrounding a fairly voluminous medulla in Dasycercus, the Phascogales, three species of Sminthopsis and *Dasyuroides byrnei,* the last having the broadest cortex of the group. *Sminthopsis crassicaudata,* the Planigales and both species of Antechonomys show a broad cortex and a reduced medulla.

In Myrmecobius the left adrenal tends to be removed from the left kidney and is usually situated slightly cranial to it and between it and the vena cava. There is, however, a certain amount of attachment to the kidney. The right gland is removed from the right kidney and is attached to the vena cava—only loosely, however. Both glands are well developed, possessing a broad cortex with the three typical cortical zones.

The Peramelidae is divisible into two groups based principally on the position of the left adrenal. All members, with one exception, show a typical, dual, vena cava —kidney attachment for the right adrenal. *Perameles myosura notina* and *P. nasuta* and *Choeropus castanotis* show the left adrenal attached to the antero-mesial border of the left kidney, but Thalacomys, Isoodon and *P. gunni* show the removal of the left adrenal from its attachment with the left kidney, to its firm connection with the point of junction of the vena cava and left renal vein.

All species exhibit a thick cortex in the gland, although it is thinnest in *Isoodon obesulus*.

In Notoryctes the right adrenal is attached principally to the vena cava, the left principally to the kidney. The cortex was very thick in each case.

In Coenolestes the right adrenal was attached principally to the vena cava, the left to the left kidney.

The Adrenal in the Phascolarctinae

The members of this sub-family are characterized chiefly by the intimate attachment of the right adrenal gland to the liver. The chief characteristic of the structure of the gland is the thin cortex and very large medulla and the fact that the cortex is definitely fasciculated and the zona glomerulosa and zona reticularis are present and generally speaking are well developed. The exception is Phascolarctus, which not only does not show the intimate attachment of the right adrenal to the liver, but in addition possesses a fairly thick cortex.

THE ADRENAL IN THE PHALANGERINAE

The right adrenal of nearly all the sub-family Phalangerinae examined show some attachment to the vena cava, many of them intimately. They also show an incipient attachment to the liver. In Trichosurus the adrenal is strongly attached to the vena cava, into which it opens by three ostia; in addition it is enclosed and in many cases completely taken up by the liver substance. All the species examined, except Trichosurus and Phalanger, show a relatively broad cortex and reduced medulla. Trichosurus shows a thin rim of fasciculated cortex and a very voluminous medulla; therefore it is typically one of the Phascolarctinae. Phascolarctus, as far as its adrenal position and structure are concerned, is more typically a member of the Phalangerinae than of its own sub-family.

From the evidence of adrenal position and structure it would appear that Trichosurus, Pseudochirus and Petauroides constitute one distinct group of the Phalangeridae, and Phascolarctus, Acrobates, Distoechurus, Dromicia, Eudromicia, Phalanger, Dactylopsila and Petaurus constitute another group.

In *Tarsips spencerae,* the honey mouse, the right adrenal rests in a small depression of the liver, but is attached only loosely, the left adrenal is associated with the corresponding kidney. The adrenal was well developed with a thick cortex which was differentiated but not completely into the three cortical zones.

ADRENALS IN THE POTOROINAE

The attachments of the adrenals in the specimens of Bettongia examined were clearly to the corresponding kidneys, but in Potorous, the attachment was not so much to the kidneys as to the vena cava, thereby indicating, perhaps, different lines of descent. In Aepiprymnus also, according to Sonntag, the attachment of the glands is to the antero-mesial borders of the corresponding kidneys.

The adrenals of all the specimens of this sub-family examined showed a broad, well-formed cortex, marked

into three zones and each zone possessing the appropriate histological structure. Thus the adrenals are in a high state of development in these animals.

Adrenals in the Macropodinae

This sub-family is divisible into two well-marked groups based on the attachment of the right adrenal gland.

In Lagorrchestes and Peradorcas the right adrenal is intimately attached to the vena cava. In Onychogale, Dendrolagus, Setonyx and various specimens of Macropus examined, the adrenal is attached definitely and intimately to the right kidney. This would then indicate that the first two members of this sub-family probably descended from an animal in which the tendency was for the adrenal to become attached to the kidney—that is, probably a more primitive group. Thus taking this single anatomical character as a criterion it would seem that the members of the sub-family Macropodinae had a diphyletic origin.

Adrenals in the Phascolomyidae

The members of this family show a right adrenal attachment which is chiefly to the right kidney, but tends also to an attachment to the vena cava. The cortex was very large and was zoned and fasciculated.

It is not proposed to discuss the adrenals in the Edentates (which show a well-defined gland) and a number of other groups of the Eutheria which have been investigated by various authors, but brief reference will be made to the adrenal in the groups examined by me.

The Ungulates and Carnivora show a well-defined adrenal with large differentiated cortex.

I have examined a very large number of rodent adrenals, and find right through the group a striking constancy in position and structure of the adrenals.

Almost without exception, the glands are typically situated on the antero-mesial borders of the corresponding

kidneys, to which they are intimately attached. This also applies to the bats.

A certain variation occurs in the position of the right adrenal in the lemurs and tree shrews, but when we come to the Primates proper we find the adrenal, a dorso-ventrally flattened cap over each kidney, to which it is intimately attached. In the case of man there is a strengthening attachment also, in the case of the right gland, to the vena cava.

It is of very great importance that Osman Hill has shown that in the adrenal glands of foetal and young Primates there is a special area of cortex identical with the famous foetal cortex of the human adrenal discovered by Elliott and Armour and others in 1911. This area in the gland is in the nature of a hyperplasia of the inner region of the cortex, which is present in the adrenal of the foetus and gradually atrophies after birth, being replaced by adult cortex.

SUMMARY OF THE ADRENAL FROM MONOTREME TO MAN

In the Monotremes the glands are loosely attached to the corresponding kidneys, but although macroscopically they are indistinguishable from higher forms, microscopically they show lack of definite mammalian structure, they are apparently still in a state of flux. There is, however, in the Marsupials a much greater variety in adrenal form and position.

From the Marsupials upwards we find the same constancy in adrenal structure and a very definite inconstancy in adrenal attachments. In the majority of the orders the gland is well developed in three diversions and is usually intimately attached to the corresponding kidney with also a tendency towards attachment to the vena cava. Then when we come to the monkeys and apes and man we find the adrenal flattened, but its attachments remain generally the same. Man and apes are the later products of an erect, or semi-erect posture, and possibly

this flattening of the adrenal is due to the pulling on the attachments of the gland in response to the necessity of the organs to reach a fresh state of equilibrium.

If we look at the group Marsupialia from the point of view of adrenal position and structure we find that the results suggest certain relationships between the Marsupial groups. This does not mean that the foot and tooth structure, as evidences of evolution, as suggested by Bensley, are ignored. The adrenal work might be described as the phylogeny of a single character, and Zimmerman (1934) has stated "the phylogeneticist of single characters does by no means maintain that the single characters developed at all isolated, independent of the species phylogenetical connections, any more than the cytologist says that nuclear division takes place isolated, independent of the rest of the ontogenetical events."

Therefore if we trace the evolution of Marsupials as shown by the adrenal gland positions, we find, firstly, a group of terrestrial forms in which the adrenals were loosely attached to their corresponding kidneys (much as they are in Echidna) began to climb trees and in doing so found it necessary to adopt the upright posture, even if only during the short time in which they were climbing the trunk of the tree. Immediately would come a need for definite fixation of organ position, including that of the adrenal.

In the case of the left adrenal, which, associated with the left kidney, is placed much more caudad in the body cavity and well beyond the posterior border of the liver, there were but two ways of making itself firm. Firstly, attachment to the vena cava if the kidney was close enough to the vein and / or if not, simply making stronger and firmer its attachments to the kidney.

With the right adrenal, it was, however, a different matter. The anterior portion of the right kidney which bore the gland was either close to or actually overlain by the posterior border of the liver, and, generally speaking, this kidney is closer to the vena cava than the left kidney,

so that on the impetus being given by the adoption of an arboreal life the right adrenal was able to cling to the vena cava, the right kidney or the posterior border of the liver. In some cases it made certain and attached itself very intimately to two or three of these sites.

If after a short arboreal period the animal again took to a terrestrial life, we would find the terrestrial forms with an indefinite series of right and probably also of left adrenal positions. Such a form amongst the Marsupials would be *Dasycercus cristicauda*. A further arboreal phase, such as Bensley suggests for the Marsupialia, would then tend to fix the adrenal positions which had been initiated in the first arboreal phase, and we would, therefore, in this case obtain Dasyures with right adrenal very firmly attached to the vena cava; Pseudochirus, Trichosurus and Petauroides with the right adrenal intimately attached to the liver and in addition forms with the adrenals intimately attached to the kidney, such as Phalangista (Distoechurus).

From the fact that the Macropidae, which may be regarded as the final terrestrial phase of the Marsupialia, contain forms in which the attachment of the right adrenal is to the vena cava (Peradorcas) and others in which the gland is attached to the kidney, it is apparent that they may have developed from a Phalangerine form in which both positions were capable of developing. Dromicia, for example, would be such a form. Presumably the upright posture of the members of the Macropidae have resulted in the perfecting of the attachment of the right adrenal, the commonest attachment of the gland being to the right kidney. In the tree kangaroos the adrenals are attached to the kidneys.

If we examine other groups of mammals apart from the Marsupials we obtain some interesting results.

Firstly in the rodents, the firm attachment of both right and left adrenals to the corresponding kidneys, with no indication of accessory attachment, is of interest, and this arrangement being characteristic of every rodent exam-

ined, with the exception of *Hydromys caurinus,* and the rabbit, indicates a surprising constancy in adrenal position after the variety met with in the Marsupialia.

In the Chiroptera, every bat examined, without even one exception, showed the typical firm supra-renal position of the adrenals. Again a surprising constancy.

In the Carnivores examined, with the possible exception of the dog, where a certain amount of variation exists in the position of the gland, again the same result, and the Ungulates similarly. In the members of the Insectivora examined by the present author—the Tupaiids, we find a certain variation in right adrenal position. In the lemurs we find the position of the glands much more constant. Then in the Anthropoidea, without exception, we find the adrenals attached in a similar fashion to the corresponding kidneys, as is found in the rodents and bats, and finally, in man, the position of the glands is the same, and in addition there is a strong fibrous attachment of the right gland to the vena cava, the continued upright position now apparently requiring extra support. With an adrenal attachment firmly to the kidney and in addition to the vena cava, the kidney itself would in this way obtain additional support.

The constancy of adrenal position in the majority of the mammalian orders would seem to indicate that somewhere has been inculcated in the early history of these forms a fairly long arboreal phase which has resulted in the fixation of the adrenals very firmly to the corresponding kidneys.

The bats must obviously have had a long arboreal phase in virtue of their brachial specialization for flight, and we find the adrenals very firmly fixed to the corresponding kidneys. We find, too, in the rodents a similar fixation of adrenal position, and also in Carnivores.

Huxley (1880) predicted from a study of recent mammals that the remote common ancestor of the various placentrals would be found to be small insectivorous forms, and the American paleontologist Osborn agrees.

Zittel points out that certain extinct families of the division "Lipotyphla" of fossil Insectivora show affinities with the modern Primates and Carnivores and rodents. Of the present-day representatives of the Insectivora—the Menotyphla representative, Tupaia, shows variation in right adrenal position.

We may assume therefore in the light of the evidence adduced that from some primitive Eocene arboreal Insectivores were evolved groups of animals in which the adrenals were very firmly attached to the kidneys. Of these races one may have taken to the ground and became the rodents. That its adrenal position was not absolutely fixed was shown by the presence of species possessing a departure in adrenal position.

Another group given off in time became the Carnivores with the adrenals also fixed. Then presumably a division occurred, one branch of which began to specialize itself for flight, and so we have the bats, a very long arboreal existence having fixed the adrenal position very definitely. Another branch persisted in its arboreal life and developed eventually to man through either monkeys and ape-like ancestors, as certain authors assert, or through Tarsius-like forms, as Wood-Jones has suggested. In Tarsius the right adrenal is firmly attached to the right kidney and the left similarly, but a lobe of the liver extends caudally along the post-caval vein, surrounding and partly enclosing the gland which fits into a little pocket in its substance. Thus Tarsius has developed an additional right adrenal attachment. Apart from the growing down of the liver lobe which appears to be a later modification, the position of the adrenals makes the derivation of man from a Tarsian-like form quite feasible, but also the adrenal position equally supports the origin of man from anthropoid-like forms. In fact, the adrenal position makes the origin of man, on one hand, and monkeys and apes, on the other, from a form having an adrenal position similar to that of Tarsius quite a reasonable hypothesis.

Adrenal position gives us no hint as to the possible relationships of the Marsupials with the remainder of the Mammalia. Both the Marsupials and the Insectivores show variation in adrenal position, which might perhaps indicate a certain amount of affinity. In Triassic times we find two groups of animals, the Allotheria and the Dromatheriidae, the first showing similarities with the Insectivores and the second with the Polyprotodont Marsupials. Still later in the Jurassic the Allotheria were still present, but the Dromatheriidae had commenced to radiate off groups of Marsupials. The Insectivores and Marsupials therefore represent the two divisions of an ancient stem form, and both, in virtue of their arboreal habits, developed a certain constancy in adrenal position in the forms which radiated from them. Owing to the Cretaceous isolation of the Marsupials in Australia, they were able to survive with little struggle for existence against more modern forms and they persist to-day as primitive remnants of an ancient era. From the fact that the Marsupials still show such a variety of adrenal attachments and the most successful mammalian groups show a great constancy in attachment of the adrenal, particularly to the kidney, it would appear that one of the many factors in the success of a race of animals in the struggle for existence might be a firm attachment of its adrenals to the corresponding kidneys. The rodents show this attachment, and to-day they are one of the most successful of the mammalian groups, exceeding all the other orders of mammals in the number of their genera and species. The bats also show this adrenal constancy, but specialization in other directions has hampered them.

Monkeys, apes and man show a similar intense association of the adrenals with the corresponding kidneys, and who will doubt that man is a successful race of animals at least as far as his ability to overrun and mismanage the earth is concerned.

The fixation of the adrenal glands, as well as other internal organs, such as the kidneys, would undoubtedly be

of service to an animal adopting an arboreal life, the mechanical perfection of a firmly attached organ would no doubt aid the animal in its attempts to gain agility, which it would gradually do as the neopallial areas of the brain associated with sight and touch developed to a greater extent. In the foregoing I have endeavored to trace mammalian evolution, using the adrenal gland. If the story told by this gland varies from the accepted facts I do not wish to flatly contradict these facts; I simply present the evidence and my interpretation of it, which may or may not be correct. For example, most evolutionists hold that the rodents did not have an arboreal ancestry.

There is apparently a very close relationship between the adrenal gland and the brain, and I would suggest in view of the evidence that follows that, since the development of Primates in general and of man is a function of the progressive development of the brain, the origin of man and apes from lower mammals has been primarily dependent upon the efficient organization of the adrenal gland, particularly in the foetus.

The evidence on which this statement is based consists of:

Firstly, in the adrenals of the foetal Primates and man an hypertrophied area of cortex occurs—the foetal cortex. This does not occur in the lower mammalian forms.

Secondly, Goldzieher (1934) has shown that extracts of the adrenal cortex (containing the hormone "cortin") which are capable of maintaining life in adrenalectomized animals possess remarkable effects on the blood lipoids. It causes them to fall—in other words—lipoidal substances previously circulating in the blood are now fixed in the tissues. Omura (1930) found that prolonged administration of cortical hormone resulted in substantial increases in weight due to fixation of the lipoidal material in the tissues.

There is not time to adduce all the evidence in favor of the control of the lipoid metabolism by the adrenal cortex, but there appears little doubt that this is the case.

In the lower mammals where there is no overgrowth of the adrenal cortex to give a "foetal cortex" the brain remains small; in the Primates, where a large "foetal cortex" occurs, a large convoluted brain is built up. Consequently, until mammals developed an adrenal, capable in the foetus of dealing with the high lipoidal metabolism required for the myelinization of a large well-convoluted brain—such a brain could not form.

If we examine cases of children born with practically no brain (anencephalic monsters) we find practically no trace of "foetal cortex" in the adrenal gland.

The work of Alpers and Haymaker (1934), in which they find in the early embryo the axones of the brain cells have no sheaths, is very important. They find that as the foetus grows, fat and lipoids are deposited in the neuroblasts and that these subsequently form themselves into myelin. Thus if the brain is to become myelinated there must be an adequate supply of lipoidal materials deposited there. The adrenal cortical hormone appears to be able to produce that result.

The facts quoted then appear to suggest that the adrenal gland is very intimately associated with the origin of man and primates from lower mammals. This suggestion then shows points of similarity with Bolk's famous endocrine hypothesis of evolution, but while Bolk (1921) suggests the development of man from a precocious foetal ape, I postulate only an origin of man, apes and monkeys from some pro-primate, as a result of certain endocrine influences—one of the most important being that of the adrenal cortex.

LITERATURE CITED

Alpers and Haymaker
1934. *Brain*, 57: 193.

Arnold
1866. *Virchow's Arch.*, Vol. 35.

Balfour
1878. "Monograph on the Development of the Elasmobranch Fishes." London.

Basir
1931. *Jour. Anat.*, 66: 628.

Bolk
1921. *Lancet*, September, 1921, p. 588.
Bourne
1935. *Australian Journal of Experimental Biology and Medicine*, 8: 113.
Elliott and Tuckett
1906. *Jour. Physiol.*, 34: 332.
Elliott and Armour
1911. *Jour. Path. and Bact.*, 15: 482.
Gaskell
1912. *Jour. Physiol.*, 44: 59.
Giacomini
1902. "Gabinetto di Zoologia ed anatomia Comparata," Univ. Perugia, Siena.
1912. *R. Accad, delle Scienze dell Instituto di Bologna*, Less. 26. Maggio 1912. Bologna. Quoted by Swale Vincent.
Goldzieher
1934. *Endocrinology*, 18: 179.
Harley
1858. *Lancet*, June, 1858, 551.
Hill, W. C. O.
1930. *Jour. Anat.*, 64: 479.
1933. *Jour. Anat.*, 68: 19.
Huxley
1880. *Proc. Zool. Soc.*, p. 645. "Sci. Memoirs," Vol. IV, p. 457.
Kolmer
1918. *Arch. Mikr. Anat.*, 91: 1.
MacKenzie and Owen
1919. "The Glandular System in Marsupials and Monotremes."
Omura
1930. *Folia Endocrinolog. Japon.*, 5: 119.
Poll
1906. Harting's "Handbuch der vergleich u. exper. Entwick. Wirbel," Jena, Bd. 3, T. 1, p. 443. Quoted by Swale Vincent.
Vincent, Swale
1924. "Internal Secretion and the Ductless Glands," London.
Zimmerman
1934. AMER. NAT., 68: 381.

SHORTER ARTICLES AND DISCUSSION

HYBRID VIGOR IN MAIZE

E. W. LINDSTROM has published in this journal (1935) a so-called "test of the Ashby hypothesis of heterosis," which calls for some comment. The present writer has made two contributions to the problem of hybrid vigor in maize (1930, 1932). Neither of these contributions contains any hypothesis, but certain data are presented, the interpretation of which is self-evident.

The data were obtained from experiments in which populations of two inbred lines of maize and the F_1 between them were grown side by side in the same environment. The populations were sampled during growth over a period of 83 days, and from these samples the relative growth rates expressed as dry-weight increase were obtained.

Size-heterosis was apparent from the outset, and after 70 days' growth the dry weight of the hybrid was four times that of the heavier parent. Nevertheless, analysis of the data showed that the relative growth rate of the hybrid did *not* exceed that of its parents, but the higher parental growth rate was inherited as a complete dominant.

The final size of a plant is the resultant of the initial size of its primordia and the relative growth rate. Since in relative growth rate the hybrid had no advantage over its parents, size-heterosis must have been due to an initially bigger primordium. In other words, if the rate of compound interest in the hybrid is no bigger, its *capital* of dividing cells must be higher.[1] The simplest way to measure this capital is to dissect the primordia (plumule and radicle) from the embryo and weigh them. When this was done it was found that the hybrid embryos were bigger than those of either parent, and the size-heterosis observed in these experiments was due solely to the maintenance of this initial advantage in primordial size. The experiments were repeated in Chicago with other strains of maize, and have since been repeated with beans and six strains of tomatoes, with the same results.

[1] It must be emphasized that this does not refer to capital of food materials, of which the maize has an enormous excess, so that any variation is negligible. It refers to "biological capital" of dividing cells in the primordium.

The life cycle of a plant may be divided arbitrarily into three periods: (i) from fertilization to the setting of seed; (ii) from germination to the onset of flowering (a period determined usually by the number of nodes formed below the flowering axis); (iii) an indeterminate senescent period from the onset of flowering to the end of the life cycle. The author's work shows that size-heterosis in the strains of plants investigated is *not* due to any greater metabolic activity in period ii, but is caused solely by a bigger product of primordia at the end of period i. Data on the behavior of hybrids in period iii have been collected, but have not yet been published. It is obvious, however, that size-heterosis in period ii cannot be explained by any events in period iii.

In his paper Lindstrom makes the false assumption that a greater growth rate in the third period may explain the hybrid vigor which the author found in the second period. He says (page 320): "The inconsistency between Ashby's results and the above data may rest on the fact that Ashby's data were gathered from the grand period of growth . . . whereas the present experiments involved the entire growth period of the plants. . . . It may well be that the vigorous F_1 hybrid makes its greater relative growth . . . at the later stages of its history."

The author has abundant evidence to show that F_1 plants behave differently from their parents at the end of the growth cycle, but the evidence throws no light whatever on the cause of size-heterosis in the grand period of growth.

In the author's opinion the conventional hypothesis of hybrid vigor is challenged by the events in the second growth period. If dominance of linked factors is invoked to explain the bigger primordial capital in the embryo, it cannot explain how the metabolic rates in the hybrid (assimilation, respiration, growth rate) remain no greater than the parental rates. Even if the hybrid continues growing after its parents have stopped, there still remains to explain the early size-heterosis on a genetic basis; and Lindstrom adduces no evidence which is relevant to this problem.

One other point in Lindstrom's paper calls for comment. He questions the determining effect of primordial capital on final size, and subjects the "hypothesis" to a "specific test." In this test neither relative growth rate nor embryo size are measured. Instead, the "capital" of the hybrid plants is reduced by cutting

off their leaves above the growing point; and despite this mutilation the hybrids exceeded their parents in final size and in yield.

It is obvious that no relevant evidence can be obtained by removing indiscriminately the organs of a plant as "capital." There is no comparison between "capital" as number of cells in the primordium and "capital" as the amount of non-meristematic leaf material. Had Lindstrom removed the flower buds, the "capital," in his sense, would have been reduced negligibly, but the yield would have fallen to zero. That removal of the photosynthetic organs stimulated vegetative vigor is in accordance with the classical work of Kraus and Kraybill (1918). It is a physiological response; and rather than being inconsistent with the author's work, it is irrelevant to that work.

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LITERATURE CITED

Ashby, E.
1930. *Ann. Bot.*, 44: 457–467.
1932. *Ann. Bot.*, 46: 1007–1032.
Kraus, E. J., and H. R. Kraybill
1918. *Oregon Agr. Col. Exp. Sta Bull.*, 149.
Lindstrom, E. W.
1935. AMER. NAT., 69: 311–322.

THE STRUCTURE OF THE CHROMOSOMES OF THE SALIVARY GLAND OF DROSOPHILA MELANOGASTER

SINCE the revival of interest in the chromosomes of Dipteran salivary glands, there has been considerable speculation concerning the significance of chromatic bands, capsules, rows of chromioles and interbandular material in these chromosomes. Particular genes have been associated with definite portions of the chromosomes, but whether the gene is located in the chromatic or achromatic portions has not been satisfactorily demonstrated (Koltzoff, 1934; Painter, 1934; Bridges, 1934; Muller, 1934; Doyle and Metz, 1935). Information in this connection has been obtained from some preliminary cytological investigations on the Drosophila salivary chromosomes.

If the chromosomes are treated with a saturated solution of carmine in 45 per cent. aqueous butyric acid they have a diameter about one and a half times that of aceto-carmine-fixed chromo-

somes. If left sufficiently long (2 to 3 weeks) in the butyric-carmine solution, each of the typical heavy bands becomes converted into a transverse row of chromioles. On being returned to aceto-carmine, the diameter of the chromosome slowly becomes reduced and the chromioles gradually approach each other and eventually present the appearance of a chromatic band though the individual chromioles remain distinguishable.

There is another interesting difference between such chromosomes and those of an ordinary aceto-carmine preparation. Rows of clear vesicles appear in the achromatic or interbandular regions throughout the chromosome. These vesicles are not in line with the chromioles along the long axis of the chromosome, but appear between the longitudinal rows of chromioles. Upon careful examination it can be seen that the vesicles are the unstained regions between the lightly staining strands connecting chromioles of adjacent bands to form a series of longitudinal cables. In short, the constituent chromonemata making up each homologue of the paired chromosomes are brought into relief by this treatment. In ordinary aceto-carmine preparations vesicles are sometimes seen, but never as clearly as in those treated as indicated above. It seems quite likely that on treating with aceto-carmine after butyric-carmine some constituent of the chromonema is removed by solution so that the residual strands appear to be more clearly separated from one another. This is supported by the observation that the interbandular material stains more heavily with butyric-carmine than with aceto-carmine, and upon being treated several times with the two alternately, the interbandular material and the chromatic material in the cytoplasm become considerably lighter. The chromosome does not have the reticulate structure described by Metz and Gay (1934) for Sciara since there is but a single strand connecting any one longitudinal series of chromioles.

Chromosomes treated with proprionic-carmine show similar tendencies but to a less marked extent.

If eggs be treated with x-rays ten to twelve hours after they are laid (25° C.) and then incubated until the larvae are about to pupate, examination of the salivary chromosomes of such larvae shows occasional vacuoles in the interbandular regions. These are larger than the vesicles previously described, occupying one half of the diameter of a single homologue. There also appear deficiencies in the chromatic bands, half a band being miss-

ing in one of the homologues. If older larvae are irradiated the induced achromatic sections are smaller. Usually the deficiencies in the chromatic bands appear in each of two adjacent bands. It is possible that in the case of the deficiencies in apparently single bands two bands are really involved, but have not been sufficiently separated in the preparation of the chromosome to appear optically as two.

The appearance of interbandular deficiencies suggests that there is a self-reproducing unit in these regions which may be inactivated by x-rays. The relatively high frequency with which pairs rather than single chromatic bands are affected indicates that the chromatic deficiency may be produced by the inactivation of a single unit in the chromonema located between the pairs of bands. The observation of Bridges (1934) that bands of particular types tend to occur in pairs is of interest in this connection. Since there is no absolute correlation between the chromatic and interbandular deficiencies, we may conclude that there are at least two units or one unit with two separable functions in the portion of the chromonema between the chromioles.

Three-day-old larvae (25° C.) have been given as much as 20,000r. without any visible effect on the salivary chromosomes. The effects described for 10- to 12-hour larvae were obtained with doses of 5,000r. At higher doses only a very few larvae survived till the fourth day. The observed chromosome abnormalities are therefore not the result of a direct effect of the radiation upon the chromosomal material already elaborated, but upon the chromosomal agents initiating the reactions which produce these materials.

If eggs are irradiated with 5,000r. 6 to 8 hours (25° C.) after they are laid, most of the salivary chromosomes show one or more translocations. In addition there are the types of abnormalities observed in irradiated 10- to 12-hour larvae. However, no translocations are produced in the latter. This is taken to indicate that the last normal mitosis occurs at about 6 to 8 hours after the egg is laid. Subsequently there are chromonematic divisions but no disjunction of the divided chromonemata. This is in agreement with the observation on Gasteria meiotic chromosomes (Marshak, 1935) that there are no obvious translocations in the metaphase and anaphase following the irradiated prophase, but that there are chromosomal attachments which upon being broken would give rise to translocations. (At doses below 500r. these

attachments appear between chromatids of the same pair of homologues, but at higher doses there are interchromosomal attachments as well.) A series of glands from larvae irradiated with 50r. to 500r. at 6 to 8 hours (25° C.) showed no translocations.

The chromatic and interbandular deficiencies occur with low frequency, only occasional chromosomes in some of the glands showing them, similarly, in Gasteria induced chromosome attachments and breaks are much more frequent than chromatic deficiencies (achromatic spots in the chromonema) (Marshak, 1935). We may conclude therefore that the fundamental structure of the chromosomes is essentially the same in both forms. More data are necessary before an exact quantitative comparison can be made. However, if the gene be identified with the self-reproducing unit of the chromonema, the results thus far obtained indicate that in the chromosomes of the Drosophila salivary gland it is located on the chromonema in the region between chromioles or chromomeres.

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LITERATURE CITED

Bridges, C. B.
1934. *Rec. of the Genetics Soc. of Amer.*, 3: 35.
Doyle, W., and C. W. Metz.
1935. *Proc. Nat. Acad. Sci.*, 21: 75.
Koltzoff, N.
1934. *Science*, 80: 312.
Marshak, A.
1935. *Proc. Nat. Acad. Sci.*, April.
Metz, C. W., and E. H. Gay
1934, *Science*, 80: 595.
Muller, H. J.
1934. *Rec. of the Genetics Soc. of Amer.*, 3: 48.
Painter, T. S.
1934. *Genetics*, 19: 448.

ISOLATION AND GROWTH CHARACTERISTICS OF THE "ZOOCHLORELLA" OF *PARAMECIUM BURSARIA*

THE "green bodies" associated with certain protozoa, sponges and metazoa have attracted the attention of biologists from time to time. Their relationship to chlorophyll-bearing forms was

first suggested by von Siebold (1849). Thirty years later DeBary (1879) introduced the term "symbiosis" into biological literature, and since then there has been much speculation as to whether the relationship of these algae and their host is one of true symbiosis, commensalism or parasitism. Brandt (1882) introduced the term "Zoochlorella" to designate these algae, but later (Brandt, 1883) withdrew the newly established generic name, since Entz (1881–1882) had reported that the algal cells from crushed *Stentor* were capable of developing independently. Bütschli (1882) suggested that Entz's cultures probably represented free-living ingested algae, rather than true "Zoochlorellae," and Pringsheim (1928) expressed the same opinion. Bütschli recognized that the green forms associated with *Frontonia leucas* did not develop in the usual manner of free-living algae, and although Beijerinck (1890) pointed out the similarity of the "Zoochlorellae" of *Hydra, Stentor* and *Paramecium* to his newly described, free-living *Chlorella vulgaris,* he actually referred to the "Zoochlorellae" infecting these forms as *Chlorella* (*Zoochlorella*) *conductrix* Brandt. He was unable to grow them independently of their hosts and suggested that possibly the green chromatophores had become modified as a result of symbiosis. LeDantec (1892) suggested that the "Zoochlorella" of *Paramecium bursaria* was perhaps identical with Beijerinck's strain of free-living *Chlorella vulgaris.* LeDantec, however, did not culture the algae independently. Oehler (1922) sought to investigate the symbiosis involved by first experimentally infecting a colorless strain of *P. bursaria* with a free-living *Chlorella* and then eliminating the alga from its host. Although he was able to do this and grow the alga independently again, Oehler was justly criticized by Pringsheim (1928) on the ground that he was dealing with an artificial infection. Hood (1927) was unsuccessful in his attempts to culture the alga of *Frontonia leucas.* Pringsheim was unable to culture independently the algae of any of his strains of *Paramecium bursaria,* nor was he able to infect a colorless strain of the same ciliate with free-living *Chlorella.* This author gives a critical discussion of the work on cultivation of "Zoochlorellae" and on the basis of his researches concludes that they are exceedingly difficult, if not impossible, to culture.

Recently (Loefer, 1934) a strain of *Paramecium bursaria* has been grown bacteria-free and its associated alga cultivated independently on agar slants. Some of these cultures have been sent

to Professor E. G. Pringsheim's laboratory at Prag for identification and further study. Preliminary reports indicate that he has no difficulty in subculturing the strain. The species, however, does not correspond to the free-living *Chlorella vulgaris*, but resembles more nearly *C. ellipsoidea*.

CULTURE AND GROWTH CHARACTERISTICS

In an aging *Paramecium bursaria* culture, the infusoria die off gradually, leaving the algae dominant. When material from such a culture is streaked or plated on 1 per cent. starch or nitrate agar, small green colonies appear after 3 to 6 days of incubation in light at room temperature. Under such conditions the strain can be subcultured without difficulty if the agar is prepared with the proper mineral solution; a strain has been maintained in this manner since January, 1934.

Considerable difficulty was experienced with the first cultures because the algae often formed only small colonies which became colorless and died in the course of a week or two. Further investigation indicated that the concentration of the mineral medium was of vital importance. When the agar was made up with a mineral solution such as used for *Chlorogonium* (Loefer, 1934a), growth was completely inhibited, as it also was when the agar was prepared with pure distilled water. When tap water was used to dissolve the agar (1 per cent.), growth was excellent. Analyses of tap water (New York City) showed that its mineral constituents were about 1/100 as concentrated as those in the solutions previously used. The following synthetic solution was accordingly substituted for tap water and proved to be quite successful:

$NaCl$	0.003 gm
$CaSO_4$	0.015 "
$MgSO_4$	0.0045 "
KNO_3	0.0013 "
$FeCl_3$	0.0002 "
Distilled water	1 liter

Growth was also tested on different agar media containing various organic compounds. Nutrient agar (Difco) was generally less suitable than starch or nitrate agar (Difco), and growth was apparently better when either 0.5 per cent. dextrose or 0.2 per cent. sodium acetate was added to the medium. The hydrogen-ion concentration of these media was adjusted to pH 6.8–7.0 with N/1 NaOH or HCl.

The importance of the concentration factor in the culture of *Paramecium bursaria* is discussed elsewhere (Loefer, 1936). From many of these experiments it was evident that *P. bursaria* is less resistant to increasing concentrations of organic nitrogen and carbon compounds than its associated alga. For example, 0.2 per cent. sodium acetate was lethal for *Paramecium,* although the algae throve on/in a similar medium. The alga again showed a higher resistance in tryptone, proteose-peptone and dextrose cultures. In a series of experiments concerning the effect of ethyl alcohol on different protozoa (Loefer and Hall, 1936) it was observed that the "Zoochlorella" of *P. bursaria* is able to withstand concentrations of alcohol as great as 3 and 4 per cent., while a 2 per cent. concentration marks the vital limit for *Chilomonas* and *P. bursaria.*

Hydrogen-ion concentration appears to be a significant factor affecting growth. For example, in a growth series on *P. bursaria,* the number of ciliates in cultures at pH 6.3 and 7.2, as determined by counting, was fairly equal. However, when the number of algae per cc was determined with a haematometer, the cultures at pH 7.2 contained five to six times as many as those at pH 6.3. Although pH 8.2–8.4 was the limit of life of *P. bursaria* in these experiments, the algae were very abundant in these media, indicating that the vital alkaline limit of these green forms is higher than that of their host.

The results of further studies on this independent strain of "Zoochlorella" should determine its taxonomic status and the degree of evolutionary modification which may have resulted from its infusorian association. Whether the alga infesting the green *Hydra* represents a different species remains to be determined. Efforts to cultivate it independently have so far proven futile. When the nutrition of colorless alga-bearing forms and that of their independently cultured "Zoochlorellae" is studied comparatively, our knowledge of the symbiosis involved in these associations will be greatly extended.

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LITERATURE CITED

Beijerinck, M. W.
1890. *Bot. Zeit. Jahrg.,* 48: 725, 740, 757, 781.
Brandt, K.
1882. *Arch. f. Anat. u. Physiol., Physiol. Abt.* 125.
1883. *Mitt. a. d. Zool. Stat. Neapel,* 4: 191.

Bütschli, O.
1880–82. "Klassen und Ordnungen des Tierreiches." Bd. 1, III.
DeBary, A.
1879. "Die Erscheinung der Symbiose." Strasburg.
Entz, G.
1881–82. *Biol. Zentralbl.*, 1: 646.
Hood, C. L.
1927. *Biol. Bull.*, 52: 79.
LeDantec, F.
1892. *Ann. Inst. Pasteur*, 6: 190.
Loefer, J. B.
1934. *Science*, 80: 206.
1934a. *Biol. Bull.*, 66: 1.
1936. *Jour. Exp. Zool.*, 72: 3.
Loefer, J. B. and R. P. Hall
1936. *Arch. f. Prot.* (in press).
Oehler, R.
1922. *Arb. a. d. Staatsinst. f. exp. Ther. u. d. Georg Speyer-Haus*, 15: 5. Jena.
Pringsheim, E. G.
1928. *Arch. f. Prot.*, 64: 289.
von Siebold, C. T.
1849. *Zeitschr. f. wiss. Zool.*, 1: 274.

THE RELATION BETWEEN b AND k IN SYSTEMS OF RELATIVE GROWTH FUNCTIONS OF THE FORM $y = bx^k$

RELATED types of organisms frequently exhibit differences in the proportions of certain parts. When the relative growth function $y = bx^k$ is applied to the growth of these parts, four possible situations may arise. The differences in proportions may be brought about by differences in the absolute size of the organisms, with b and k the same for each type; by differences in b, with k remaining fixed; by differences in k, with b remaining fixed; or by differences in both b and k.

In the last case, where both b and k vary, it is important to know whether these two parameters are functionally related and, if so, what the nature of the relationship is. This problem has been attacked in only a few instances. Hersh (1931), in an investigation of the relative growth of dorsal and ventral lobes of the eye in different genetic stocks of bar-eyed Drosophila, found that there exists between b and k a relationship which is approximately expressed by a decreasing exponential function of the form

$$b = Be^{-rk} \quad \text{.....(1)}$$

In a later investigation on phylogenetic relative growth of skull width and skull length in different genera of titanotheres (Hersh, 1934) the same relationship was found to hold. Paulian (1934) remarks that in the case of the brain size/body size relation in several species of Crustacea and teleost fish, b seems to vary inversely with k, but the data are inadequate for the determination of a more precise mathematical law. An inverse relationship has been observed also between the values of b and k characterizing the relative growth of head length and body length in seasonal varieties of *Daphnia longispina* (calculated from the data of Volterra, 1926).

It is evident that in all these cases b and k are related in essentially the same manner. If equation (1) is assumed to be strictly valid, we then have a family of curves whose relationship to one another is given more precisely in the following theorem:

Given the system of linear equations

$$\log y = \log b_i + k_i \log x \quad \text{.....(2)}$$

$i = 1, 2, 3, \ldots$ the necessary and sufficient condition that these equations have a common solution $(x = x_0, y = y_0)$ is that

$$b = Be^{-rk}$$

where $r = \log x_0$ and $B = y_0$.

To prove the necessary condition we need merely consider x and y in the equation $\log y = \log b + k \log x$ as having the fixed values x_0 and y_0, while b and k vary. We then have

$$\begin{aligned} \log b &= \log y_0 - k \log x_0 \\ &= \log B - rk. \end{aligned}$$

where $B = y_0$, $r = \log x_0$. It follows immediately that

$$b = Be^{-rk}.$$

The sufficiency of the condition can be shown as follows. Consider any two equations of the set (2):

$$\begin{aligned} &\log y = \log b_i + k_i \log x \\ &\qquad \text{.....(3)} \\ &\log y = \log b_j + k_j \log x \end{aligned}$$

By hypothesis

$$\begin{aligned} \log b_i &= \log B - rk_i \\ \log b_j &= \log B - rk_j \end{aligned}$$

Substituting these values in equations (3) we have

$$\log y = \log B + k_i (\log x - r)$$
$$\log y = \log B + k_j (\log x - r)$$

Substracting the second of these equations from the first, we obtain

$$(k_i - k_j)(\log x - r) = 0$$

from which it follows that $k_i = k_j$ or $\log x = r$. If $k_i = k_j$, then $b_i = b_j$, for b is a single-valued function of k, and hence the curves coincide. If $k_i \neq k_j$, then we must have $\log x = r$. It is then readily ascertained that $y = B$, and the equations (2) must therefore have the common solution $y = B$, $\log x = r$.

Equation (1) thus implies that the graphs of equations (2) on a double logarithmic grid are a family of straight lines intersecting at a common point, with coordinates (r, $\log B$). For the length/width relationship of the skull in titanotheres Hersh (1934) finds that $B = 301.8$, $r = 6.218$. Hence we should expect the relative growth curves to intersect at the point (6.218, log 301.8). Inspection of his graph (Fig. 1, p. 538) reveals immediately, however, that there is by no means a common point of intersection. The same is true of his curves for the dorsal lobe/ventral lobe relation in bar-eyed Drosophila (1931, Figs. 1–8, pp. 232–233).

It follows, therefore, that equation (1) is incompatible with the actual relationship between the relative growth curves. This apparent contradiction is not surprising, however, if we bear in mind the fact that both the relative growth function and equation (1) are empirical approximations. It has been frequently pointed out that the same set of data can be fitted, within the limits of the probable error of the observed points, by mathematical expressions of very different types (see Enriques, 1909; Gray, 1928; Kavanagh and Richards, 1934). Hence the particular equation employed cannot be assumed to be theoretically valid without external evidence. It is dangerous, consequently, to attempt further mathematical theorization from such empirical equations.

The present case illustrates this point clearly. While it is obvious that a relationship between b and k exists, it can be said of equation (1) only that it represents an empirical approximation without theoretical validity. The biological significance of the relationship is nevertheless of importance, and the problem merits further investigation.

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LITERATURE CITED

Enriques, P.
1909. *Biol. Cent.* Bd. 29, S. 331–352.
Gray, J.
1929. *Brit. Jour. Exp. Biol.*, 6: 248–274.
Hersh, A. H.
1931. *Jour. Exp. Zool.*, 60: 213–248.
1934. AMER. NAT., 68: 537–561.
Kavanagh, A. J. and O. W. Richards
1934. AMER. NAT., 68: 54–59.
Paulian, R.
1934. "Contribution a l'étude de la croissance relative du système nerveux central. Mémoire presenté à la Faculté des Sciences de l'Université de Paris." Les Presses Universitaires de France. No. 544.
Volterra, L.
1926. *Int. Rev. Hydrob. Hydrog.*, Bd. 15, S. 204–239.

SPECIES FROM THE GENETIC STANDPOINT

DARWIN thought that the sterility of hybrids was not a valid criterion in the determination of species because for all practical purposes it is most difficult to ascertain when fertility ends and sterility begins. He held that the only criterion by which it can be decided whether a doubtful form is to be ranked as a species or as a variety is *the opinion of naturalists having sound judgment and wide experience.* It is doubtful if very much has been learned in the meantime which supersedes this very common sense concept of species in general. Working taxonomists as a rule still proceed along conservative and constructive lines, and my experience indicates that they are remarkably accurate. It seems that biologists in general should be more grateful for and appreciative of the orderly successes, than vexed at and disparaging of such shortcomings as are inevitably incident to the intricacies and difficulties of this essential occupation. Authentic taxonomists may be regarded as the indispensable sergeants of the army biological.

However, from the view-point of the geneticist, or the otherwise experimental evolutionist, the line between fertility and sterility of hybrids is of considerable importance. The experimental production or observation of the development of species that will not cross back with the progenies of the common ancestor or, if so, produce only sterile hybrids, is probably the eventual goal. This was the consummation eagerly awaited back in the days of the

sagacious Bateson. It may be recalled that there was a tone of impatience, or even irritation, in some of the judgments passed on Bateson's implicit and categorical challenge. Yet it could hardly be maintained that there was anything unfair in the demand for just that achievement, and so a number of investigators undertook to meet it.

In my opinion this challenge has been met successfully by E. W. Lindstrom, E. B. Babcock and others, and in more than one way. It appears that varieties, or species, of tomatoes, *Crepis* and other plants have been produced by critical experimentation that do not breed back with other progenies of the common ancestor. What they are called does not matter, whether varieties or species; the bridging of the gap is the essential accomplishment. Perhaps investigators will henceforth be able to achieve something more than the analyses and syntheses of characteristics within and among readily intra- and inter-breeding varieties, however valuable such work is known to be. If this crucial feat can be accomplished successfully and consistently and over a wide range of plants and animals, it may be considered a very high measure of the effectiveness of genetics as the instrument for solving some of the problems of the transmutation of species, and evolution. Perhaps this is about as far as the geneticist, as such, need be concerned with the problem of species.

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